Program Nr: 1 from 2002 ASHG Annual Meeting

**Isolation of a gene for common forms of stroke.** *S. Gretarsdottir[1], G. Thorleifsson[1], A. Manolescu[1], S. Jonsdottir[1], S.Th. Reynisdottir[1], H.M. Gudjonsdottir[1], Th. Jonsdottir[1], H. Gudmundsdottir[1], G. Gudmundsson[1], S. Sveinbjornsdottir[2], E.M. Valdimarsson[2], F. Jakobsson[2], U. Agnarsson[3], V. Gudnason[3], G. Thorgeirsson[3], M. Gurney[1], M. Frigge[1], A. Kong[1], K. Stefansson[1], J. Gulcher[1].* 1) deCODE Genetics, Reykjavik,Iceland; 2) National University Hospital, Reykjavik, Iceland; 3) Icelandic Heart Association Heart Preventive Clinic, Reykjavik,Iceland.

Stroke is one of the most complex diseases of man with several subtypes as well as secondary risk factors such as hypertension, hyperlipidemia, and diabetes, which in turn have genetic and environmental risk factors of their own. We have mapped the first major locus for common forms of stroke to 5q12, designated as STRK 1 (Gretarsdottir et al. 2002, AJHG 70:593-603). The lod score at this locus met the criteria for genome-wide significance (multipoint allele sharing lodscore of 4.40, p-value of 3.9 X 10-6 ). By extensive fine-mapping we narrowed down the most promising region for harbouring a stroke susceptibility gene to a segment less than 4.1 Mb. We have sequenced this segment and identified genes in the region. Based on association analysis using a dense set of microsatellite markers and SNPs, typed for 1200 patients and 650 unrelated controls, we have identified one of these genes as a strong candidate gene for common forms of stroke. This gene encodes for an enzyme expressed in vascular tissue and in cell types that may play a key role in atherosclerosis. We will present the gene, the association results and results from ongoing functional work.

Program Nr: 2 from 2002 ASHG Annual Meeting

**Genomic DNA Insertions and Deletions Occur Frequently Between Humans and Nonhuman Primates.** *K.A. Frazer, C. Chen, D. Hinds, K. Pant, N. Patil, D.R. Cox.* Perlegen Sciences, Mountain View, CA.

Comparative DNA sequence studies between humans and nonhuman primates will be important for understanding the genetic basis of the phenotypic differences between these species. Previous human-chimpanzee DNA comparison studies focused on analyzing short (~ 1-10 kb) aligned orthologous sequences have indicated that at the nucleotide level humans and chimpanzees are ~ 98.6% identical. However, the number of sequences that are present in humans and absent in chimpanzees or vice versa due to genomic rearrangements, has not yet been examined. We have compared ~ 27 Mb of chimpanzee DNA with orthologous human chromosome 21 sequences by hybridizing chimpanzee sequences amplified by long-range PCR to human high-density oligonucleotide arrays. Our comparative study identified 57 genomic rearrangements (deletions and insertions ranging in size from 0.2 to 8.0 kb) in the chimpanzee DNA relative to the reference human DNA. These rearrangements, of which ~ 35% are present within genes, have occurred in the genomes of both humans and chimpanzees, as well as other nonhuman primates. The observed DNA rearrangements provide natural starting points for focused investigations of qualitative and quantitative gene expression differences between humans and other primates. Moreover, our results suggest that small genomic rearrangements may play a larger role in genome evolution than previously recognized.

Program Nr: 3 from 2002 ASHG Annual Meeting

**Substantial long-term clinical improvement in dogs with mucopolysaccharidosis VII following neonatal retroviral vector-mediated gene therapy.** *M. Haskins[1], J. Melniczek[1], L. Xu[2], M. Weil[1], T. O'Malley[1], P. O'Donnell[1], V. Knox[1], G. Aguirre[3], M. Sleeper[1], N.M. Ellinwood[1], S. Volk[1], H. Mazrier[1], R. Mango[2], J. Zweigle[1], J. Wolfe[1], K.P. Ponder[2]*. 1) School of Veterinary Medicine, Univ Pennsylvania, Phila., PA; 2) Washington Univ School of Medicine, St. Louis, MO; 3) College of Veterinary Medicine, Cornell Univ, Ithaca, NY.

Mucopolysaccharidosis (MPS) VII is caused by deficient activity of b-glucuronidase (GUSB). The phenotype of MPS VII dogs, similar to human patients, includes dysostosis multiplex, growth retardation, corneal clouding, and cardiac valve abnormalities. Five MPS VII dogs were injected on day 2-3 of life with 3.1x10E9 infectious units (IU)/kg of an amphotropic retroviral vector (RV) containing the human a1-antitrypsin promoter, canine GUSB cDNA, and woodchuck hepatitis post transcriptional element. GUSB staining of liver biopsies at 3 months demonstrated 2.8% transduced hepatocytes, resulting in 195 U/ml (73% of normal) GUSB in serum, a level maintained for 15 months in the 3 dogs still being followed. Another neonatal MPS VII dog received 2.5 mg/kg of hepatocyte growth factor (HGF) prior to the injection of 12x10E9 IU/kg of the RV. GUSB staining of a liver biopsy demonstrated 19% transduced hepatocytes, resulting in 16,800 U/ml (63-fold normal) serum GUSB at a year and a half. Untreated MPS VII dogs weigh 51% of normal as adults. The 3 RV-treated dogs and HGF/RV dog achieved 90% and 95% of normal body weight as adults, respectively. Untreated affected dogs cannot stand or ambulate beyond 6 months of age. In contrast, all treated dogs could walk and run for the duration of evaluation (6-18 months). Radiographs of the hind limbs demonstrated that the treated dogs had significant ($p=0.04$ to $<0.001$) decreases in most abnormalities compared with age-matched, untreated MPS VII dogs. The treated dogs corneas remained clear and they had no detectable heart murmurs or mitral valve thickening. We conclude that a simple neonatal IV injection of RV results in high-level and long-term expression of GUSB from the liver that has a marked effect upon major clinical manifestations of disease in MPS VII dogs. DK54481, RR02512, DK48028, EY13132, RR T32-07063.

Program Nr: 4 from 2002 ASHG Annual Meeting

**Fanconi Anemia Complementation Group D2 (fancd2) knockout mice display microphthalmia and an increased susceptibility to breast and ovarian cancer.** *S. Houghtaling[1], C. Timmers[1], S. Meyn[2], M. Noll[1], C. Reifsteck[1], S. Olson[1], S. Jones[4], M. Finegold[3], M. Grompe[1]*. 1) Dept of Mol and Med Gen, OHSU, Portland, OR; 2) Dept of Mol and Med Gen, Univ of Toronto, Toronto, Canada; 3) Dept of Path, Baylor College of Medicine, Houston, TX; 4) Umass, Worcester, MA.

Fanconi anemia (FA) is an autosomal recessive disorder characterized by bone marrow failure, congenital birth defects, and an increased incidence of cancer. At least 7 complementation groups exist and recently BRCA2 has been identified as a member of the FA pathway. FANCD2 has been shown to act distally to the other FA proteins and is mono-ubiquitinated during S-phase and following DNA damage. This results in its co-localization in nuclear foci with BRCA1 and RAD51 and suggests that the FA pathway may control homologous recombination during S-phase. To further investigate the in vivo function of the FA pathway we have disrupted fancd2 by homologous recombination. Fancd2 -/- mice display phenotypes not observed in other FA knockout mice. In the C57/Bl6 background 80% of mutant mice have microphthalmia. Fancd2 -/- mice also display a more severe decrease in germ cell number than fanca, c, or g -/- mice. In addition mispairing of chromosomes during pachytene of meiosis was seen. Significantly, 2/5 female fancd2 -/- mice older than 14 months had breast or ovarian cancer. Similar to FA patients and other FA mouse models, fancd2 -/- mice exhibit in vivo ionizing radiation (IR) sensitivity and cellular sensitivity to DNA crosslinking agents. FANCD2 was recently reported to be a substrate of the ATM kinase and implicated in controlling an S-phase checkpoint in response to IR. Therefore, primary fancd2 -/- fibroblasts were tested for the presence of radio-resistant DNA synthesis (RDS), a measure of this S-phase checkpoint. Fancd2 -/- cells did not display RDS and thus we conclude that FANCD2 is not essential for the S-phase checkpoint in primary cells and that the modest IR sensitivity of fancd2 -/- cells is not due to defective ATM signaling. Overall, the phenotype of fancd2-/- mice supports the hypothesis that the FA proteins (including BRCA2) and BRCA1 participate in a common S-phase specific DNA damage response pathway.

Program Nr: 5 from 2002 ASHG Annual Meeting

**DNA sequence-based virtual human chromosomes.** *O.A. Haas, A. Schmidt, S. Strehl.* CCRI, St Anna Children's Hosp, Vienna, Austria.

The human genome can be analyzed morphologically with microscopic and chemically with molecular genetic means. At present, it is not possible to directly compare, correlate, exchange, or jointly analyze data that are generated the one or the other way within a single platform. One essential prerequisite for such an integrated approach is the transformation of the descriptive cytogenetic and molecular cytogenetic data into a sequence format. The availability of the human sequence (http://genome.ucsc.edu/) enabled now for the first time a direct evaluation of the relationship between the sequence and the chromosomal banding pattern. Our reconstruction of the human chromosomes with electronic means revealed an astonishing concordance between these two banding patterns. This observation proofs that the "large-scale" chromosomal banding solely reflects the DNA sequence and that it is hardly modified by epigenetic factors. However, virtual chromosomes provide also a unique basis for the joint processing of cytogenetic, FISH, and molecular genetic data within a single DNA sequence-based framework. The accurate, scale-independent, and highly region-specific banding pattern can be easily adapted to that of natural chromosomes irrespective of their state of condensation. In combination with the possibility to electronically reconstruct any chromosome rearrangement with a hitherto unknown molecular precision, this may provide the basis for novel cytogenetic pattern recognition systems. Moreover, we are also able to map and display the chromosomal position of any sequence or set of sequences, including the distribution of the currently available approximately 14.000 genes. The superimposition of such a graphic interface on molecular genetic databases will thus enable the visualization of molecular events in a chromosomal fashion. In addition to virtual gene mapping, such a tool will enable the display of micro array-derived DNA and gene expression profiles. Finally, it also remains possible to integrate more fuzzy-defined morphological events, such as those obtained with conventional cytogeneticis and with a variety of FISH methods, including comparative genome hybridization (CGH) and comparative expressed sequence hybridization (CESH).

Program Nr: 6 from 2002 ASHG Annual Meeting

**Tuncating Neurotrypsin mutation in autosomal recessive non-syndromic mental retardation.** *F. MOLINARI, M. RIO, F. ENCHA-RAZAVI, M. VEKEMANS, T. ATTIE-BITACH, A. MUNNICH, L. COLLEAUX*. INSERM U393, et Departement de Genetique Medicale,Hopital Necker-Enfants Malades, PARIS, FRANCE.

Mental retardation (MR), defined as an intelligence quotient (IQ) below 70, is the most frequent cause of serious handicap in children and young adults. Moderate to severe MR (IQ<50) concerns 1% of the population and its prevalence increases up to 2-3% if mild MR is included (50 Here we report the identification of the first gene involved in autosomal recessive isolated MR. Homozygosity mapping in an inbred eastern algerian family with four mentally retarded children (3 girls and 1 boy) localized the disease causing gene on chromosome 4q24 between markers D4S1564 and D4S402. This interval encompasses the PRSS12 gene (also known as BSSP-3) encoding a brain-specific serine protease named neurotrypsin. We identified a 4 base-pair deletion (del ACGT1391-1394) within the coding sequence that segregates with the disease. This mutation is likely a null allele as it is predicted to result in a shortened protein lacking the catalytic domain. *In situ* hybridization experiments showed that neurotrypsin is highly expressed in human fetal brain structures involved in learning and memory.

Our results suggest that anormal proteolysis of extra cellular matrix macromolecules may interfere with normal development of the nervous system, providing therefore new insight into the understanding of the pathophysiological bases of mental retardation.

Program Nr: 7 from 2002 ASHG Annual Meeting

**A Kinesin Heavy Chain (*KIF5A*) mutation in Hereditary Spastic Paraplegia (*SPG10*).** *M.T. Kloos[1], E. Reid[2], A. Ashley-Koch[3], L. Hughes[1], S. Bevan[2], I. Svenson[1], P.C. Gaskell[3], A. Dearlove[4], M.A. Pericak-Vance[3], D.C. Rubinsztein[2], D.A. Marchuk[1]*. 1) Department of Genetics, Duke University Medical Center, Durham, NC; 2) Department of Medical Genetics, University of Cambridge, Cambridge UK; 3) Center for Human Genetics, Duke University Medical Center, Durham, NC; 4) MRC UK HGMP Resource Centre, Cambridge, UK.

The Hereditary Spastic Paraplegias (HSPs) are a genetically heterogeneous (17 loci to date) group of neurodegenerative disorders characterized by progressive spasticity and weakness in the lower limbs. Their common pathology is characterized by axonal degeneration of motor and sensory neurons that is maximal at the distal ends of the longest axons of the central nervous system. Although the genes for six HSP loci have been identified, no unifying theme has emerged to explain the disease pathogenesis. We have identified a missense mutation (N256S) in the motor domain of the neuronal kinesin heavy chain gene *KIF5A*, in a family with HSP. The mutation occurs in the original *SPG10* family at an invariant asparagine residue which, when mutated in orthologous kinesin heavy chain motor proteins, prevents stimulation of the motor ATPase by microtubule-binding. Mutations in kinesin orthologues in various invertebrate and vertebrate species lead to phenotypes resembling HSP. The kinesin motor powers intracellular movement of membranous organelles and other macromolecular cargo along microtubule tracks extending from the neuronal cell body to the distal tip of the axon. Since the neurons innervating the lower limbs have axons of up to 1 meter in length, they represent the most extreme example of the difficulties of proper intracellular trafficking. The gene for the most common form of HSP (*SPG4*) encodes spastin, recently shown to interact dynamically with microtubules. Taken together, these data suggest that the underlying pathology of *SPG10* and possibly other forms of HSP may involve perturbation of neuronal anterograde axoplasmic flow, leading to axonal degeneration, especially in the longest axons of the central nervous system.

Program Nr: 8 from 2002 ASHG Annual Meeting

**Mitochondrial DNA Point Mutations in Patients with MNGIE.** *Y. Nishigaki[1], R. Marti[1], M. Lin[2], M. Hirano[1]*. 1) Neurology, Columbia University, New York, NY; 2) Neurology, Cornell University, New York, NY.

Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder due to mutations in the gene encoding thymidine phosphorylase (TP), and is associated with multiple deletions and depletion of mitochondrial DNA (mtDNA). Metabolism of thymidine and deoxyuridine are altered in MNGIE. We have hypothesized that increased thymidine and deoxyuridine cause imbalances of mitochondrial nucleotide pools which in turn leads to mtDNA abnormalities. To identify point mutations of mtDNA, we sequenced tRNA and protein coding genes of cultured MNGIE fibroblasts, and identified three heteroplasmic T-to-C mutations; all were 5'-AAAT to 5'-AAAC. To screen for additional 5'-AAAT to 5'-AAAC transitions, RFLP analyses were performed on DNA extracted from the cultured fibroblasts, lymphocytes, and tissue samples from MNGIE patients. Fibroblast mtDNA from 6 MNGIE patients had 6 heteroplasmic 5'-AAAT to 5'-AAAC mutations. Three of the mutations were present in all 6-cell lines with heteroplasmic levels ranging from 1 to 41%. These mutations were also present in lymphocytes and tissues from MNGIE patients. The mutations were absent in controls and unaffected carriers of TP mutations. Sequencing of the two hypervariable segments (HV1 and HV2) that are thought to be hot spots for mitochondrial mutations in the D-loop was performed to screen for mutations at non-AAAT sites. PCR-amplified HV segments of two patients and two controls were subcloned, and 92-95 clones of each PCR-fragment were sequenced. Four additional 5'-AAAT to 5'-AAAC heteroplasmic mutations were identified in HV1 and HV2. In addition, the following mutations were identified: 5-AAT to AAC, 5-GGAGT to 5-GGAGC, 5'-AAAT to 5-AAAA (two sites), 5-AAATT to 5-AAAAA, and del-T at 5-AAAAAATTT. Two 5'-AAAT sites did not show mutations. In summary, we have found 12 T-to-C heteroplasmic transitions in the MNGIE. All of the mutations were preceded by at least three purines, and 9/12 were preceded by 5'-AAA sequences. Our results indicate aberrant thymidine metabolism in MNGIE produces somatic point mutations of mtDNA at specific sequence motifs.

Program Nr: 9 from 2002 ASHG Annual Meeting

**Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome.** *D. Beltran-Valero[1], S. Currier[2], A. Steinbrecher[3], J. Celli[1], H. Kayserili[4], L. Merlini[5], D. Chitayat[6], W. Dobyns[7], B. Cormand[8], T. Voit[3], C. Walsh[2], A. Lehesjoki[8], J. Cruces[9], H. van Bokhoven[1], H. Brunner[1]*. 1) Human Genetics, University Med Centre Nijmegen, Nijmegen, The Netherlands; 2) Neurogenetics, Beth Israel Deaconess Medical Center, Boston; 3) Pediatrics and Pediatric Neurology, University Hospital Essen, Germany; 4) Pediatrics, Istanbul Medical Faculty, Istanbul University, Turkey; 5) Neuromuscular Pathology, Istituto Ortopedico Rizzoli, Bologna, Italy; 6) Obstetrics and Gynecology, Mount Sinai Hospital, University of Toronto, Canada; 7) Human Genetics, Neurology and Pediatrics, University of Chicago, Chicago; 8) Folkhalsan Institute, University of Helsinki, Finland; 9) Biochemistry, University Autonoma Madrid, Spain.

Walker-Warburg syndrome (WWS) is an autosomal recessive developmental disorder characterized by congenital muscular dystrophy and complex brain and eye abnormalities. A similar combination of symptoms is presented by two other human diseases, Muscle-Eye-Brain disease (MEB), and Fukuyama congenital muscular dystrophy (FCMD). While the genes underlying FCMD (Fukutin) and MEB (POMGnT1) have been cloned, loci for WWS have remained elusive. A genome-wide linkage analysis in ten consanguineous WWS families revealed the existence of at least three WWS loci. Subsequently, we adopted a candidate gene approach in combination with homozygosity mapping in 15 consanguineous WWS families. As POMGnT1 encodes an O-mannoside N-acetylglucosaminyltransferase, we analyzed the possible implication of O-mannosyl glycan synthesis in WWS . The analysis of the locus for O-mannosyltransferase1 (POMT1), revealed homozygosity in 5 of 15 families. Sequencing of POMT1 gene revealed causative mutations, including nonsense and frameshift mutations, in 6 out of the 30 unrelated WWS patients. Immunohistochemical analysis of muscle from patients with POMT1 mutations corroborated the O-mannosylation defect, as judged by the absence of glycosylation of a-dystroglycan. The implication of O-mannosylation in MEB and WWS suggests new lines of study in understanding the molecular basis of neuronal migration.

Program Nr: 10 from 2002 ASHG Annual Meeting

**Transgenic mice expressing mutant a-synuclein (A53T) develop neurological disorder and pathology.** *H.X. Deng[1], R. Fu[1], H. Zhai[1], M.C. Dal Canto[2], T. Siddique[1].* 1) Neurology Dept, Northwestern Univ, Chicago, IL; 2) Pathology Dept, Northwestern Univ, Chicago, IL.

Parkinsons disease (PD) is the second most common neurodegenerative disorder characterized by akinesia/bradykinesia, rigidity and loss of normal postural reflexes, and hyperkinetic signs such as tremor. Dopaminergic neuronal cell death in substantia nigra and intracytoplasmic Lewy bodies containing a-synuclein are the hallmarks of PD. The etiology of PD is not well known. Two mutations in a-synuclein gene (A53T and A30P) were identified in a small number of familial PD cases. To investigate if and how mutations in a-synuclein lead to PD, we developed transgenic mouse lines expressing wild-type and A53T of human a-synuclein under control of neuronal-specific enolase (NSE) promoter. Among 10 lines expressing A53T, three lines developed neurological disorder by 12~17 months and died 2~3 weeks later. The affected mice initially showed reduced movement activity. With the disease progression, one line developed resting tremor and another line developed action tremor (video available). At the end stage, mice showed lower body temperature and became paralyzed. Pathological analysis suggested broad neurogenerative changes in central nerve system including astrocytosis. Punctuated a-synuclein-positive inclusions were broadly present in CNS. The mice expressing human wild-type a-synuclein remain unaffected so far. Our results suggest that A53T of a-synuclein is toxic to neuronal cells and this mouse model may provide a useful tool for further investigation of the pathogenesis of a-synuclein mediated neurodegeneration including PD and also for in vivo therapeutic drug testing.

Program Nr: 11 from 2002 ASHG Annual Meeting

**Beta-synuclein transgenic mice show significant reductions in alpha-synuclein protein expression levels.** *P. Limprasert[1], I.V.J. Murray[2], D.W. Tsuang[3], J.B. Leverenz[3], V.M.Y. Lee[2], J.Q. Trojanowski[2], A.R. La Spada[1].* 1) Dept Lab Medicine, Univ Washington, Seattle, WA; 2) Dept Path & Lab Med, Univ Pennsylvania, Philadelphia, PA; 3) VA Puget Sound Health Care System, Seattle, WA.

Parkinson's disease (PD) is a movement disorder characterized by tremor, rigidity, and bradykinesia. The pathological hallmark of PD is the Lewy body, an intra-cytoplasmic inclusion composed of a-synuclein. Alteration in the conformation of a-synuclein may be central to the pathogenesis of PD, as inheritance of mutant forms of a-synuclein has been linked to PD. b-synuclein is a closely-related member of the synuclein family, and is co-expressed with a-synuclein in synaptic terminals. Recent data suggest that b-synuclein can inhibit a-synuclein aggregation and neurodegeneration when co-expressed in transgenic mice. To determine the role of b-synuclein in neurodegenerative disorders involving Lewy body formation, we generated lines of transgenic mice that express elevated levels of human b-synuclein protein. Visible inspection and rotarod testing of mice up to 14 months of age indicated that they display normal movement control and coordination, with a trend toward superior coordination in b-synuclein transgenic mice. Neuropathology studies showed that brains of b-synuclein transgenic mice do not develop aggregates or inclusions. Western blot analysis of brain protein extracts suggested that b-synuclein transgenic mice express reduced levels of a-synuclein. To verify this result, we performed I-125 quantification of a-synuclein protein levels with two different anti-a-synuclein antibodies, normalizing to neuron-specific enolase. For each of the b-synuclein transgenic lines analyzed, a-synuclein protein levels were reduced to 53-58% (range = 46-71%) of the a-synuclein levels measured in controls ($p < .01$). These results suggest that the two synucleins may be coordinately controlled and that the mechanism of b-synuclein protection may involve down-regulation of a-synuclein. If production of toxic a-synuclein protofibrils is a key event in PD pathogenesis, this finding supports delivery of b-synuclein to the striatum as a potentially useful therapy in PD.

Program Nr: 12 from 2002 ASHG Annual Meeting

**Antioxidant proteins TSA and PAG are candidates for early Alzheimers pathogenesis.** *M. McElroy, L. Reiter, M. Wangler, J. Trimble, E. Bier.* Dept Biol, Univ California, San Diego, La Jolla, CA.

Mutations in the transmembrane protein presenilin 1 (*PSEN1*) result in the most prevalent form of familial early-onset Alzheimers disease (FAD). FAD mutations in *PSEN1*can lead to over-production of a neurotoxic form of b-amyloid found in senile plaques. Although the cellular function of PSEN1 is uncertain, PSEN1 has been implicated in diverse cellular activities including activation of the Notch developmental signaling pathway, calcium homeostasis, and apoptosis. There is also evidence suggesting that presenilins play a role in antioxidant biology. Recently, two antioxidant proteins, TSA and PAG, were found to interact with PSEN1 in a yeast two-hybrid screen. We tested for *in vivo* interactions between these antioxidant proteins and PSEN1 by misexpressing them in *Drosophila* using the GAL4-UAS transactivation system. *Psn* misexpression gives a weakly penetrant phenotype that appears to compromise Notch signaling and results in apoptosis. When PAG and/or TSA, which generate no wing phenotype on their own, are coexpressed with Psn, a strong enhancement of the Psn misexpression phenotype is observed indicating that these proteins function in a concerted fashion. Analysis of Notch target genes indicates that coexpression of PAG, TSA, and Psn interferes with the Notch mediated activation of these genes. It has recently been shown that oxidative damage may be the most critical early step in the etiology of Alzheimers even prior to the formation of senile plaques. We tested whether TSA and PAG could alter the shortened lifespan associated with loss of function mutations in the supersoxide dismutase (*SOD*) gene. Co-expression of TSA with low levels of SOD resulted in increased longevity of *sod-* mutant flies. Six human genes homologous to TSA and PAG map within 7 different FAD candidate gene loci and should be considered strong candidate genes for FAD.

Program Nr: 13 from 2002 ASHG Annual Meeting

**Phosphorylation of ataxin-1: Cell signaling and neurodegeneration.** *M.D. Kaytor[1], E.S. Emamian[1], L.A. Duvick[1], C.E. Byam[1], S.L. Wilber[1], S.K. Tousey[1], H.Y. Zoghbi[2], H.T. Orr[1].* 1) Dept 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 disorder caused by the expansion of a glutamine repeat within the *SCA1*-encoded protein, ataxin-1. A critical factor in the pathogenesis of SCA1 is the subcellular localization and deposition of mutant ataxin-1. As phosphorylation is a means to control protein localization and degradation, we sought to determine if ataxin-1 is phosphorylated. We show that glycogen synthase kinase 3b (GSK3b) can phosphorylate serine 776 of ataxin-1 *in vitro*. Mutation of serine 776 to alanine reduces the phosphoylation of ataxin-1; furthermore, using a phospho-specific ataxin-1 antibody we show that the GSK3b inhibitor lithium reduces phosphorylation of serine 776. Co-immunoprecipitation experiments indicate that ataxin-1 can form a complex with GSK3b. This complex is disrupted *in vitro* by lithium or by mutation of either the GSK3b phosphorylation site (A776) or the putative GSK3b priming site (A780). A776[82Q] does not form inclusions *in vitro* while the ability of A780[82Q] to form inclusions is similar to that of ataxin-1 with serines at 776 and 780. To examine the role of Ser 776 and Ser 780 in SCA1 pathogenesis, we have established *SCA1* transgenic mice expressing A776[82Q] or A780[82Q]. To date we have seven A776 lines, which upon immunohistochemical analysis do not contain ataxin-1 inclusions in Purkinje cells. As these mice age we will determine if A776[82Q] can cause disease. The A780[82Q] construct is currently being injected. In summary, A776[82Q] does not form inclusions but retains partial ability to interact with GSK3b, while A780[82Q] forms inclusions but does not interact with GSK3b. Hence, characterization of the A776 and A780 transgenic mice will allow us to assess the relative importance of inclusion formation and interaction of ataxin-1 with GSK3b in SCA1 pathogenesis.

Program Nr: 14 from 2002 ASHG Annual Meeting

**Neto1, a novel synaptic putative membrane receptor associated with aberrant axonal guidance and seizures.** *D. Ng[1,2], A. Sertie[1,2], R.K. Szilard[1,2], L.V. Kalia[3], M.W. Salter[3], R.R. McInnes[1,2].* 1) Program in Genetics; 2) Dev Bio; 3) Brain and Behaviour, Hosp for Sick Children, Toronto.

To identify novel proteins required for neurodevelopment, we conducted an in silico screen and identified Neto1 and Neto2, two related proteins with similarity to the axon guidance receptors, neuropilins, & the patterning protein, tolloid. The Netos have homologous structures (two extracellular CUB domains (extracellular protein:protein association motifs), a single TM domain, and a cytoplasmic tail) and overlapping neurospecific expression. In adult, expression is prominent in layer V cerebral cortical neurons (whose axons form the corticospinal tract (CST)), limbic system, basal ganglia, pons, and retinal interneurons, as shown by in situ hybridization and a tau-lacZ reporter gene knock-in of Neto1. Neto1 expression is first seen in dorsal root ganglia at E12.5, and in the telencephalon and neuroretina at E13.5. In transfected cells, Neto1 localizes as a homomultimer to the plasma membrane. To begin to define the functions of Neto1, we identified cytoplasmic binding partners. The C-terminal PDZ binding ligand of Neto1 predicted its binding to PSD-95, a scaffold protein involved in protein assembly at the postsynaptic (PS) membrane. This prediction was confirmed by 2 hybrid assays, GST-pull downs, the presence of Neto1 in synaptosomal & PS density fractions, and co-IP of Neto1 and PSD-95. The CNS of Neto1-/- mice is histologically normal. Loss of Neto1 function leads to two distinct phenotypes: i) gene dosage-dependent apparent seizures (involuntary whole body shakes) that are more frequent, but not more severe, in Neto1-/- than Neto1+/- mice, and ii) defects in the CST axonal projections at both the pre- and post-pyramidal decussation. We conclude that 1) Neto1 & Neto2 define a novel family of putative neurospecific receptors, 2) Neto1 is a component of the PS macromolecular assembly organized by PSD-95, 3) Neto1 is essential for normal neurodevelopment, particularly axonal guidance, 4) Neto1-/- mice are likely to have defects in synaptic transmission, 5) NETO1, which maps to 18q22-23, is a strong candidate gene for human epilepsy and other disorders affecting the synapse.

Program Nr: 15 from 2002 ASHG Annual Meeting

**In-vitro analysis of Zic2 mutations indicates that holoprosencephaly results from a loss of transactivating function.** *S.A. Brown, M. Abigania, L.Y. Brown.* Columbia University, Dept.of Ob/Gyn, New York, NY.

Heterozygous mutations in the transcription factor gene, Zic2, result in holoprosencephaly (HPE). Thus far, the molecular mechanism involved in this malformation process is completely unknown. While many of the Zic2 mutations that we have encountered in HPE patients clearly result in complete loss of function (null alleles), other mutations are predicted to result in altered Zic2 proteins that possibly have diminished or altered function. In particular, many mutations have occurred in the carboxy terminus of the protein outside of the predicted DNA binding domain. At least 6 patients have had identical expansions of a carboxy-terminal alanine tract. Understanding how protein function is altered by these mutations will be of great help in dissecting the molecular details of Zic2 function.

To this end, we have analyzed 8 different Zic2 mutation alleles with respect to their activity in an in-vitro luciferase reporter assay, an in-vitro DNA binding assay and an in-vitro expression assay. We show that all HPE associated mutations result in near complete loss of activity but that in those cases that do not alter the predicted DNA binding domain, DNA binding activity is apparently normal. This implies that mutations have disrupted the transactiviating function of the Zic2 protein and that protein-protein interactions involving the carboxy-terminus (including the alanine tract) are critical for normal activity. One of the mutations, a carboxy-terminal frameshift has a strong dominant negative activity, and another deletion allele exhibits supra-normal activity. Finally, one deletion allele has lost its nuclear localizing function since the deleted protein is present throughout the cytoplasm of transfected cells. These results will be of great value in future experiments in which Zic2 expression is manipulated in experimental animals.

Program Nr: 16 from 2002 ASHG Annual Meeting

**RNA-Initiated Allelic Exclusion in Nonsense-Mediated Decay(NMD)-Deficient Mice.** *P.A. Frischmeyer[1,4], R.A. Montgomery[2,4], S.K. Cooke[2,4], D.S. Warren[2,4], C.J. Sonnenday[2,4], A.L. Guerrerio[3,4], C. Morrell[1,4], H.C. Dietz[1,4]*. 1) HHMI/Institute of Genetic Medicine; 2) Dept. of Surgery; 3) Dept. of Biophysics and Biophysical Chemistry; 4) Johns Hopkins University, Baltimore, MD.

Thymic maturation requires T-cell receptor-b (TCRb) allele rearrangement. The random nature of this process contributes to immunologic diversity but mandates that 2 of 3 events will create a premature termination codon. If a cell is nonproductive on the first attempt ($b^{-}b^{o}$), the nonsense transcripts will be degraded by NMD and the other allele will undergo rearrangement. If a cell is productive on the first try ($b^{+}b^{o}$), a process termed allelic exclusion (AE) precludes rearrangement of the other allele. Although current doctrine holds that AE requires feedback signaling from a mature TCRb chain at the cell surface, the uniform establishment of AE despite an obligate time lag between productive recombination and feedback is difficult to reconcile. We previously reported that an NMD-deficient mouse line (Tg) shows arrested maturation of developing thymocytes. While these data were consistent with a deleterious consequence for stabilized TCRb nonsense transcripts, a nonspecific toxic effect of NMD inhibition could not be excluded. Here we show that the thymic phenotype of Tg animals could be rescued by transgenic introduction of a productively rearranged TCRb allele that induces AE of both endogenous alleles, supporting the former hypothesis. Paradoxically, Tg mice show an over-representation of out-of-frame alleles, suggesting either prolonged survival of $b^{-}b^{-}$ cells or inappropriate AE with increased numbers of $b^{-}b^{o}$ cells. Fetal transgenic thymocytes demonstrated normal Db to Jb rearrangements (which are not subject to AE), but diminished Vb to DbJb rearrangements (which are). Importantly, any peptide derived from nonproductively rearranged TCRb alleles would lack domains known to be critical for signaling. These data are most consistent with a model that invokes a direct role for TCRb mRNA in the initiation of AE. Attractively, this model would also reconcile the time lag paradox inherent to prior models. These studies document the first physiologic role for NMD in higher eukaryotes.

Program Nr: 17 from 2002 ASHG Annual Meeting

**Direct involvement of the nonsense surveillance machinery in nonsense-mediated altered splicing (NAS).** *J.T. Mendell[1], C.M.J. ap Rhys[2], H.C. Dietz[1,2].* 1) Johns Hopkins U. Sch. of Med., Baltimore, MD; 2) HHMI.

Mutations that create premature termination codons (PTCs) contribute significantly to inherited disease. In addition to inducing transcript degradation through the nonsense-mediated mRNA decay (NMD) pathway, nonsense mutations can associate with alterations in pre-mRNA splicing. Multiple models have been invoked to reconcile how nonsense codon recognition (traditionally considered a cytoplasmic event) could influence nuclear pre-mRNA metabolism. First, the nucleotide substitution could directly disrupt an exonic splicing enhancer or induce a conformation unfavorable for splicing, irrespective of any influence on reading frame. Alternatively, nonsense-mediated altered splicing (NAS) could be an indirect effect that is induced upon recognition and decay of the mature transcript by NMD. The most controversial view posits that the nucleus is competent for nonsense surveillance. We used RNA interference (RNAi) in mammalian cells to examine the role of factors essential for NMD in NAS of T cell receptor-b nonsense transcripts. Inhibition of rent1/hUpf1 expression abrogated both NMD and NAS. In contrast, inhibition of rent2/hUpf2 expression did not disrupt NAS despite achieving comparable stabilization of nonsense transcripts. Furthermore, development of a novel allele-specific RNAi methodology allowed demonstration that NAS and NMD are genetically separable functions of rent1/hUpf1. Consistent with these observations, we provide evidence that rent1/hUpf1, but not rent2/hUpf2, enters the nucleus where it may directly influence early events in mRNA biogenesis. These findings establish that the effects of PTCs on mRNA stability and pre-mRNA processing result from distinct rent1/hUpf1-dependent cellular processes. Involvement of the NMD machinery in NAS provides direct evidence that disruption of reading frame *per se* can influence splice site selection and is fully consistent with the emerging view that the nucleus is capable of nonsense surveillance. These data offer the intriguing possibility that nonsense surveillance contributes broadly to the metabolism of nascent transcripts including noncoding RNAs with a physiologic absence of coding potential.

Program Nr: 18 from 2002 ASHG Annual Meeting

**Multiple Roles for the ORF1-encoded Protein in LINE-1 Retrotransposition.** *D.A. Kulpa, J.V. Moran.* Dept Human Genetics, Univ Michigan, Ann Arbor, MI.

L1s are abundant non-LTR retrotransposons that comprise ~17 % of human DNA. Most L1s are mutated and cannot retrotranspose; however, ~60 elements are retrotransposition-competent (RC-L1s). RC-L1s are 6 kb, and contain a 5' untranslated region (UTR), two open reading frames (ORF1 and ORF2), and a 3' UTR that ends in a polyadenylic acid tail. ORF1 encodes an RNA binding protein (ORF1p), whereas ORF2 encodes a protein (ORF2p) with endonuclease and reverse transcriptase activities. L1 retrotransposition requires the transcription of L1 RNA, its transport to the cytoplasm, and translation of its two open reading frames. Recent experiments indicate the L1-encoded proteins demonstrate a cis-preference and preferentially associate with their encoding transcript to form a ribonucleoprotein particle (RNP), which is a proposed retrotransposition intermediate. To complete retrotransposition, the RNP is transported to the nucleus, and the L1 RNA is reverse transcribed via target primed reverse transcription (TPRT), and integrated into genomic DNA. Here, we exploited cis-preference to follow the fate of wild type and mutant L1 proteins against a background of endogenously expressed L1s. We demonstrated that L1s containing an epitope tag at the carboxyl terminus of either ORF1p or ORF2p remain retrotransposition-competent, and that tagged ORF1p and its encoding RNA localize to cytoplasmic RNPs that are distinct from translating polyribosomes. Consistently, we identified three ORF1p mutants that block both retrotransposition and RNP formation, suggesting that RNP assembly is a necessary step for retrotransposition. Interestingly, in contrast to previous reports, we have detected ORF1p in the nucleus. We also have uncovered an ORF1p missense mutation that localizes the protein to RNPs, but is unable to retrotranspose. Together, our data strongly suggest that ORF1p is not only needed for RNP formation, but also functions at later steps in the L1 retrotransposition pathway.

Program Nr: 19 from 2002 ASHG Annual Meeting

**Transcriptional Regulation of the Lactase-phlorizin Hydrolase (LPH) Gene by Polymorphisms Associated with Adult-type Hypolactasia.** *M. Kuokkanen[1,5], N.S. Enattah[1,5], A. Oksanen[2], E. Savilahti[3], A. Orpana[4,6], I. Jarvela[4,5].* 1) Molecular Medicine, National Public Health Institu, Helsinki, Finland; 2) Herttoniemi Hospital; 3) Hospital for Children and Adolescents; 4) Laboratory of Molecular Genetics, HUCH-Laboratory Diagnostics; 5) Department of Medical Genetics, University of Helsinki; 6) Department of Clinical Chemistry, University of Helsinki, Finland.

We have determined the functional significance of the recently identified two single nucleotide polymorphisms (SNPs), C/T-13910 and G/A-22018 associated with adult-type hypolactasia by studying the LPH mRNA levels in intestinal biopsy sample with different genotypes.Intestinal biopsy sample were taken from 52 patients with abdominal complaints. Hypolactasia was diagnosed by determining lactase and sucrase activities and their (L/S) ratio. The functional effect of the C/T-13910 and G/A-22018 genotype on the expression of LPH mRNA was demonstrated in patients heterozygous for the C/T-13910 and G/A-22018 polymorphism and an informative expressed SNP located in the coding region of the LPH mRNA. RT-PCR followed by solid-phase minisequencing was used for accessing the relative expression levels of the LPH alleles using informative SNPs located in several exons of the LPH gene. Statistically significant difference between the genotypes CC-13910, GG-22018, CT-13910,GA-22018 and TT-13910, AA-22018 and lactase/sucrase activity ratio (L/S ratio) was detected. Relative quantitation of the expressed LPH alleles showed that the persistent allele represented a mean 11.5 fold difference in the relative amounts of the LPH alleles. The patient with the homozygous persistent TT-13910, AA-22018, as well as hypolactasic patients with the CC-13910, GG-22018 showed equal expression of both alleles. These findings suggest that the two SNPs C/T-13910 and G/A-22018 associated with adult-type hypolactasia are associated with the transcriptional regulation of the LPH gene. Based on these results invasive endoscopy in the diagnosis of adult-type hypolactasia might be replaced by genotyping of the associated SNPs in the future.

Program Nr: 20 from 2002 ASHG Annual Meeting

**Angiotensinogen gene polymorphism at -217 affects basal promoter activity and is associated with hypertension in African-Americans.** *S. Jain[1], X. Tang[1], Y. Agarwal[2], S. Peterson[2], C. Brown[3], J. Ott[4], A. Kumar[1]*. 1) Pathology, New York Medical College, Valhalla, NY; 2) Internal Medicine, New York Medical College, Valhalla, NY; 3) renal division, department of Medicine, SUNY Health Science Center, Brooklyn, NY 11203; 4) Laboratory of Statistical Genetics, Rockefeller University, NY 10021.

Hypertension is a serious health problem in the Western society, in particular for the African-American population. Previous studies have suggested that angiotensinogen (AGT) gene is associated with human essential hypertension in Caucasian and Japanese populations. We show here that an A/G polymorphism at -217 in the promoter of the AGT gene plays an important role in hypertension in African-Americans. We have analyzed genomic DNA from 186 hypertensive and 156 normotensive African-American subjects. The frequency of the -217A allele in hypertensive patients was 0.29 as compared to 0.19 in normotensive population which is highly significant (p= 0.0017 and OR =1.792). We also show that the nucleotide sequence of this region of AGT gene promoter binds strongly to the C/EBP family of transcription factors when nucleoside A is present at -217. In addition, we show that reporter constructs containing human AGT gene promoter with nucleoside A at -217 have increased transcriptional activity on transient transfection in HepG2 cells as compared to reporter constructs with nucleoside G at -217. Finally, we show that co-transfection of either C/EBP-alpha or C/EBP-beta increases the promoter activity of reporter constructs containing nucleoside A at -217. Since, the AGT gene is expressed primarily in liver and adipose tissue and C/EBP family of transcription factors plays an important role in gene expression in these tissues, we propose that increased transcriptional activity of the -217A allele of the human AGT gene is associated with hypertension in African-Americans.

Program Nr: 21 from 2002 ASHG Annual Meeting

**Mutations in two genes encoding different subunits of a receptor signaling complex result in identical disease phenotype with dementia and bone fractures, PLOSL.** *J. Paloneva[1], T. Manninen[2], G. Christman[2], K. Hovanes[2], J. Mandelin[3], R. Adolfsson[4], M. Bianchin[5], T. Bird[6], R. Miranda[7], A. Salmaggi[8], L. Tranebjærg[9], Y. Konttinen[3], L. Peltonen[1,2]*. 1) Dept. of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 2) Dept. of Human Genetics, UCLA School of Medicine, UCLA, Los Angeles, CA; 3) University of Helsinki; 4) University of Umeå Sweden; 5) Hospital Regional de São José Brazil; 6) University of Washington; 7) Clinica Modelo, Bolivia; 8) Instituto Nazionale Neurologico C. Besta, Milano; 9) University Hospital of Tromsø.

Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL), also known as Nasu-Hakola disease, is a globally distributed recessively inherited disease leading to death in the fourth decade, and characterized by early onset progressive dementia with bone fractures. We have earlier identified PLOSL mutations in DAP12, which codes for an activating membrane receptor component in natural killer (NK) cells and cells of monocyte-macrophage lineage. We also recognized genetic heterogeneity in PLOSL, some patients carrying no mutations in DAP12. To identify the second PLOSL -locus we analyzed families showing exclusion of linkage to the PLOSL -locus in the chromosome 19q13.1 for segregation of the marker haplotypes flanking genes that encode the polypeptides interacting with DAP12, and identified a single locus on 6q21 showing co-segregation with PLOSL. Here we complete the molecular pathology of PLOSL by identifying mutations in TREM2 in 20% of PLOSL patients, 80% carrying mutations in DAP12. On the cell membrane TREM2 forms a receptor signaling complex with DAP12 and triggers activation of the immune responses in macrophages and dendritic cells. PLOSL patients have no defects in cell-mediated immunity suggesting a remarkable capacity of the human immune system to compensate for the inactive DAP12-mediated activation pathway. Our data imply a significant role of the DAP12-TREM2 complex in the human brain and bone tissue, and provide an interesting example how mutations in two different subunits of a multi-subunit receptor complex result in an identical human disease phenotype.

Program Nr: 22 from 2002 ASHG Annual Meeting

**Mice with the ATP8B1$^{G308V/G308V}$ targeted mutation suggest disregulation of intestinal bile salt resorption as a cause of inherited cholestatic liver disease.** *L. Pawlikowska[1,2], E.F. Eppens[2], R. Ottenhoff[2], N. Looije[2], A.S. Knisely[3], W. Kramer[4], L.N. Bull[1], R.P. Oude Elferink[2], N.B. Freimer[5]*. 1) UCSF Liver Center, University of California, San Francisco, CA; 2) Laboratory of Experimental Hepatology, Academic Medical Center, Amsterdam, The Netherlands; 3) King's College Hospital, London, UK; 4) DG Metabolic Diseases, Aventis Pharma Deutschland GmbH, Germany; 5) Departments of Psychiatry & Human Genetics, University of California, Los Angeles, CA.

Mutations in *ATP8B1 (FIC1)*, a broadly expressed P-type ATPase that probably functions as a plasma membrane aminophospholipid translocase, result in disorders of bile secretion, but the etiological role of FIC1 in liver disease is unclear. We investigated the systemic impact of FIC1 loss by generating Fic1$^{G308V/G308V}$ mutant mice. In contrast to human patients homozygous for this mutation, Fic1$^{G308V/G308V}$ mice had unimpaired bile secretion and no liver damage, but showed perturbed bile salt (BS) homeostasis including elevated serum BS levels. Feeding the *Fic1* mutant mice a BS-supplemented diet caused dramatic accumulation of serum BS (mutants vs. wildtypes: 448 ± 270 vs. 2.0 ± 1.1 mM; $p<0.0001$) and mild liver injury due to excessive intestinal BS absorption, demonstrated by high retention of orally administered labeled BS in mutant mice on BS diet (28 ± 4.5% vs. 1.8 ± 0.4%; $p<0.0001$). This retention was 50% reduced by a specific inhibitor of ileal BS uptake (BI-1, Aventis), indicating that hyperabsorption in *Fic1* mutant mice is due in part to impaired downregulation of the intestinal BS transporter ASBT. Since Western blotting revealed similar ASBT protein levels in wildtype and mutant mice under all conditions, such regulation may happen at the level of ASBT activity or localization. Our results show that Fic1 deficiency disables regulation of intestinal BS resorption, but the low toxicity of the murine BS pool probably protects *Fic1* mutant mice from liver damage. The *Fic1* mutant mice reveal the importance of regulation of ileal BS resorption for BS homeostasis and suggest that such defective regulation may be a primary insult in the etiology of human FIC1-related liver disease.

Program Nr: 23 from 2002 ASHG Annual Meeting

**Tissue-specific DNA methylation correlates with brain-specific imprinting of the Angelman gene, *UBE3A*.** *Y-H. Jiang, J. Bresslller, Q. Liu, A.L. Beaudet.* Dept. of Moelcular & Human Genetics, Baylor Col Medicine, Houston, TX.

The *UBE3A* gene maps to human chromosome 15q11-q13. Deficiency of maternal expression of *UBE3A* causes Angelman syndrome, a disorder characterized by severe mental retardation, seizures, and absence of speech. Although tissue-specific paternal silencing of *UBE3A* in human brain and mouse cerebellar Purkinje cells and hippocampal neurons has been demonstrated, little is known about the mechanisms underlying the tissue-specific imprinted expression. There is evidence that the regulation of imprinted expression for *UBE3A* involves 1) cis regulatory elements within the imprinting center (IC) 2) differential DNA methylation within the imprinted domain and 3) antisense transcription overlapping sense transcription at the *UBE3A* locus. We have attempted to identify a differential methylation region that may be associated with imprinted expression of the UBE3A gene. The CpG island at the 5' end of *UBE3A* was consistently unmethylated with no allele- or tissue-specific differential methylation. However, tissue-specific differential methylation region was found at the 3' end of *UBE3A* (*UBE3A* 3'-TS-DMR). The *UBE3A* 3'-TS-DMR was found to be completely methylated in the liver, heart, and cultured lymphoblasts, but almost completely unmethylated in cerebellar cortex and vermis and partially unmethylated in various areas of the cerebral cortex and hippocampus. The variation in DNA methylation in different areas of the cerebral cortex is very extensive, with intensity of the methylated allele prominent in Brodmann areas 20, 22, 44, and 45, but less prominent in 4, 10, 17, and 41. The 3'-TS-DMR is embedded within sequences highly similar to Alu repeats, but it does not fit exactly into any of the known families of Alu repeats. Sequence analysis revealed two tandem CTCF-like binding sites within the 3'-TS-DMR. The functional significance of the CTCF binding sites is being assessed by an *in vitro* protein-binding assay. We propose a model for the tissue-specific imprinted expression of *UBE3A* integrating the IC and tissue-specific DNA methylation within the 15q11-q13 imprinted domain.

Program Nr: 24 from 2002 ASHG Annual Meeting

**A Screening System to Detect Mutations Affecting Snrpn Gene Imprinting in ES cells.** *M. Wu, A. Beaudet.* molecular and human genetics, Baylor College of Medicine, Houston, TX.

A subset of mammalian genes is subject to an imprinting process that epigenetically marks alleles according to the parental origin. These genes are often organized into large clusters and are regulated by imprinting centers. The promoter of Snrpn gene expressed only from the paternal chromosome lies within an imprinting center (IC) that controls the imprinted genomic marks in the Prader-Willi/Angelman domain. To investigate the mechanisms of imprinting in this region, we developed a system for large-scale screening in mouse embryonic stem cells (ES) to identify mutations controlling Snrpn expression. The method incorporates a green fluorescent protein (GFP) marker, which is knocked in downstream of the Snrpn promoter. We screened for regulatory mutations through spontaneous effects, as well as by EMS or gene-trap mutagenesis. The common mechanism we found for loss of GFP-Snrpn expression was spontaneous methylation on the fusion allele. Screen for EMS mutation in ES cells led to the identification of ATG to AAG mutation at the initiation codon of Snurf-Snrpn analogous to ENU mutagenesis in mice (Hum Mol Genet 2002 Jul). Gene trap studies resulted in the isolation of two related genes implicated in the regulation of chromatin remodeling. Studies of gene expression in ES cells and transgenic mice, as well as knockout mice, are in progress to delineate the role of the two genes in transcription generally and in genomic imprinting specifically. Taken together, The GFP-based screening system makes it possible to conduct a wide survey of imprinting regulation.

Program Nr: 25 from 2002 ASHG Annual Meeting

**Extensive linkage disequilibrium and a 16.7 kb deletion of protocadherin** a**8,** a**9 and** a**10 genes are common features of the human protocadherin** a **gene cluster.** *R.M. Myers[1,2], J.P. Noonan[1], C. Caoile[2], M. Dickson[2], L. Nguyen[1]*. 1) Department of Genetics, Stanford Univ Sch Medicine, Stanford, CA; 2) Stanford Human Genome Center, Stanford Univ Sch Medicine, Stanford, CA.

Regions of extensive linkage disequilibrium appear to be a common feature of the human genome (Reich et al. 2001, Daly et al 2001). However, the mechanisms that maintain these regions are unknown. Recombination may be discouraged in repeat-rich regions due to deletions and duplications that can result from unequal crossover events. Previous measurements of LD have focused mostly on single genes. In an effort to understand whether gene density contributes to LD, we determined the degree of regulatory sequence variation in a large tandemly arrayed gene family, the human protocadherin genes on chromosome 5. These genes are expressed at synaptic junctions in the developing and adult brain (Kohmura et al 1998) and are thought to be involved in determining synaptic complexity. We sequenced the promoters of all the a protocadherin genes in 96 individuals and identified common SNPs in several of them. We found that these polymorphisms are in extensive linkage disequilibrium, forming two 48 kb haplotypes extending from the a1 through the a7 genes that are of equal frequency in Caucasians. We also determined allele frequencies and calculated linkage disequilibrium statistics for these polymorphisms in East Asians, African-American and native African populations. Our results indicate that extensive LD is an ancient feature of the a gene subcluster. We observed no similar pattern of common polymorphism or LD in the promoters of the b or g genes, indicating that extensive LD is a feature of the a protocadherin genes and not a general characteristic of the entire gene-dense cluster. We also discovered a 15 kb deletion that truncates the a8 gene and completely removes the a9 and a10 genes. This deletion appears in normal individuals from multiple populations, suggesting that it is also ancient and that a reduction in protocadherin gene number is not necessarily deleterious. Reich et al. (2001) Nature 411: 199. Daly et al (2001) Nature Genetics 29: 229. Kohmura et al (1998) Neuron 20: 1137.

Program Nr: 26 from 2002 ASHG Annual Meeting

**Holoproesencephaly due to ZIC2 mutations: Clinical, neuroradiological, and molecular studies.** *M. Muenke[1], A. Slavotinek[1], S. Odent[2], V. David[2], S. Brown[3] and Carter Centers for Holoprosencephaly and Related Brain Malformations.* 1) NHGRI/NIH, Bethesda, MD; 2) U. Rennes, France; 3) Columbia U., New York, NY.

Here we report our findings in individuals with known mutations in the most common holoprosencephaly (HPE) genes, SHH and ZIC2. A preliminary analysis of clinical data from 107 HPE patients/gene carriers from 29 unrelated families with SHH mutations did not result in a distinct phenotype. In contrast, phenotypic differences emerged when HPE patients with alterations in ZIC2 were studied. Here we describe 28 patients in 19 unrelated families with ZIC2 mutations. 1) The majority of patients had severe HPE (alobar and semilobar), although less severe forms (lobar HPE and middle interhemispheric variant) were seen in some. 2) In contrast to patients with mutations in other HPE genes, no major structural anomalies of the face (such as cyclopia, anophthalmia, ethmocephaly, cebocephaly, or clefting) were noted. In general, HPE patients with ZIC2 alterations have a normal or mildly dysmorphic face. 3) In this preliminary study, it appears that despite the severe CNS anomalies, children with changes in ZIC2 live longer than children with CNS malformations of similar severity due to mutations in SHH. 4) Two of 19 families with ZIC2 mutations had three pregnancies with anencephalic fetuses (although tissue was not available for ZIC2 analysis). 5) In contrast to SHH, the majority of ZIC2 mutations were de novo, and not present in the parents, with the exception of the alanine expansion which was inherited from the mosaic carrier father (in 3 of 3 families when both parents were available). Our preliminary clinical and neuroradiological findings are consistent with the expression pattern of ZIC2 in the dorsal neural tube (in contrast to SHH which is expressed in the ventral neural tube). Interestingly, diminished expression of Zic-2 in the mouse leads to neural tube defects as well as HPE, which is consistent with the finding of anencephaly in three fetuses of this study. The elucidation of phenotypic differences in HPE according to molecular basis will be of great help in ultimately understanding the embryologic processes that lead to HPE.

Program Nr: 27 from 2002 ASHG Annual Meeting

**Sotos syndrome is cased by haploinsufficiency of the NSD1 gene.** *N. Kurotaki[1,2], N. Harada[1,2], N. Niikawa[1,2], N. Matsumoto[1,2].* 1) Dept Human Genetics, Nagasaki Univ Sch, Nagasaki, Japan; 2) CREST, Japan Science and Technology Corporation, Kawaguchi, Japan.

Sotos syndrome (SS, cerebral gigantism, OMIM *117550) is a neurological disorder characterized by prenatal-childhood overgrowth with advanced bone age, a peculiar face with large skull, acromegalic features and pointed chin, occasional brain anomalies and seizures, and mental retardation. SS patients were estimated to have a 3.9 % risk of benign and/or malignant tumors. We isolated the NSD1 gene from the 5q35 breakpoint of a patient with a de novo balanced translocation, t(5;8)(q35;q24.1) by positional cloning. NSD1 encodes 2,696 amino acids with SET, PHD finger, and PWWP domains, and may interact with nuclear receptors (NRs). It is expressed in the fetal and adult brain, kidney, skeletal muscle, spleen, and the thymus, and faintly in the lung. Among 42 SS patients examined, we detected 4 (10 %) de novo point mutations in NSD1, including a nonsense mutation (1310C->G, S437X), a one-bp deletion (3536delA), a one-bp insertion (5998insT), and a splice-donor-site mutation (6151+1G->A). In addition, FISH analysis using BAC clones flanking the 5q35 breakpoint revealed submicroscopic deletions involving the entire NSD1 gene in 20 (67%) of 30 patients whose chromosomes were available (Nat Genet 30:365-366, 2002). These results indicate that haploinsufficiency of NSD1 is the major cause of Sotos syndrome. We are analyzing additional 31 SS patients. Novel point mutations and deletions will be presented.

Program Nr: 28 from 2002 ASHG Annual Meeting

**NDS1 gene deletion is not a frequent cause of Sotos syndrome.** *L. Clech, L. Colleaux, J. Amiel, L. Faivre, M. Le Merrer, S. Lyonnet, A. Munnich, V. Cormier-Daire.* Department of Genetics and INSERM U393, Hopital Necker Enfants Malades, Paris, France.

Sotos syndrome is an overgrowth syndrome characterized by pre and postnatal overgrowth, macrocephaly, advanced bone age and distinctive facial features. Chromosome anomalies have been reported in a few cases and recently, haploinsufficiency of the NDS1 gene has been identified in 24 cases in a series of 42 patients with Sotos syndrome (Kurotaki et al, 2002). Submicroscopic deletions were identified in 20/24 and point mutations in 4/24 patients. Based on these results, we looked for NDS1 deletions in a series of 39 patients using microsatellite markers flanking the NDS1 gene. The series included typical Sotos patients (14/39), Sotos-like patients (lacking one major criteria, 9/39), Weaver patients (6/39) and overgrowth patients with mental retardation (10/39). We found 4 NDS1 deletions in our series. Interestingly, all four deletions were of paternal origin. The four children were diagnosed as Sotos-like syndrome as they did not have any advanced bone age. Overgrowth was absent or moderate (+2.5 SD) but characteristic facial features of Sotos syndrome and macrocephaly were consistently observed. In addition, all four had severe mental retardation. These results suggest that NDS1 deletions are not a major cause of Sotos syndrome (17 % of Sotos and Sotos-like patients in our series) but are associated with significant mental retardation. Moreover, we did not find evidence for involvement of NDS1 gene in either Weaver syndrome or other overgrowth syndromes. Additional studies are required to accurately delineate the clinical phenotype of NDS1 deletions and to confirm the paternal origin of these deletions.

Program Nr: 29 from 2002 ASHG Annual Meeting

**Intragenic mutations of *NSD1* are a major cause of Sotos syndrome and Weaver syndrome but do not account for other overgrowth phenotypes.** *N. Rahman[1], J. Douglas[1], S. Hanks[1], I.K. Temple[2], H.E. Hughes[3], T. Cole[4] and The Childhood Overgrowth Consortium.* 1) Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, England; 2) Dept of Medical Genetics, Southampton, UK; 3) Institute of Medical Genetics, Cardiff, UK; 4) Clinical Genetics Unit, Birmingham Womens Hospital, Birmingham UK.

Sotos syndrome is a relatively common childhood overgrowth syndrome. Diagnosis is primarily based on the characteristic facial appearance (macrocephaly, prominent jaw, sparse hairline), together with height and OFC >97th centile. Additional features include advanced bone age and developmental delay. Congenital heart disease, scoliosis and / or cancer are present in some cases. Recently, deletions of *NSD1* were reported to be the major cause of Sotos syndrome with intragenic mutations reported in a minority of cases. We have evaluated 76 cases of childhood overgrowth for mutations and deletions of *NSD1*. The cohort was phenotypically scored into three groups prior to the molecular analyses. Group 1 (38 cases) were typical of Sotos syndrome; Group 2 (12 cases) were Sotos-like but with some atypical features; Group 3 (19 cases) did not have Sotos syndrome. We also analysed 7 cases with Weaver syndrome. In total, we identified intragenic *NSD1* mutations in >40% of the cohort. The majority of these mutations result in premature truncation of the protein. In contrast to the initial report, deletion of the whole gene was uncommon. There was a strong correlation between presence of *NSD1* mutation and clinical phenotype: 70% of Group 1 (classic Sotos) individuals had *NSD1* mutations whereas no Group 3 (non-Sotos overgrowth) individuals had *NSD1* mutations. Three individuals with Weaver syndrome had *NSD1* mutations, indicating that this condition is allelic with Sotos syndrome, at least in some cases. We conclude that intragenic mutations of *NSD1* are the major cause of Sotos syndrome and are responsible for some Weaver syndrome cases, but do not account for other childhood overgrowth phenotypes. Email: nazneen@icr.ac.uk.

Program Nr: 30 from 2002 ASHG Annual Meeting

**Computer-based recognition of syndromic faces.** *B. Horsthemke[1], D. Wieczorek[1], H.S. Loos[2], C. von der Malsburg[2].* 1) Inst Humangenetik, Universitatsklinkum, Essen, Germany; 2) Inst Neuroinformatik, Universitat, Bochum, Germany.

The ability to recognize a specific facial pattern in patients with a genetic syndrome indicates that there is consistency in the phenotypic expression of the affected genes between unrelated individuals. Facial resemblance is often used to make a clinical diagnosis, but it takes a particular skill to extract a disease-specific pattern from a facial apperance that is also influenced by family background and environment. Objective techniques for assessing craniofacial morphology by anthropometric measurements on patients or photographs can be used to determine distances between facial landmarks, but are insufficient to describe the overall facial pattern. We have investigated whether a pattern recognition program that uses pixels (grey-level values) can recognize syndromic faces. 2D photos were taken with a standard digital camera. For preprocessing of the pictures (256x256 pixels) we used a Gabor wavelet transformation, because receptive fields of simple cells in the primary cortex are of similar shape as Gabor kernels and this approach has been successfully used for checking the identity of an individual. For each pixel, a vector with 40 complex coefficients (jet) was generated. For the purpose of this study, each face was automatically labelled with 48 nodes, and the jets attached to each node of a face were then compared to the jets of all nodes at the same fiducial points of every face in the data base (bunch graph). Classification was based on a majority decision of all analysed nodes of a face (jet voting). Analysis of 32 innerfacial nodes from 55 frontal view photographs of patients with mucopolysaccharidosis type III (n=6), Cornelia de Lange (n=12), fragile X (n=12), Prader-Willi (n=12), and Williams-Beuren syndrome (n=13) revealed correct syndrome recognition in 42/55 (76%) of the patients. In another four patients (7%), the correct and an incorrect diagnosis scored equally well. Compared to random assignment (20%), the recognition rate was very high. Our results suggest that it will be feasible to develop a program which may aid the clinical diagnosis of genetic syndromes and the study of genetic variation of facial patterns.

Program Nr: 31 from 2002 ASHG Annual Meeting

**Reversal of sex ratio in fetal anticonvulsant syndrome associated autistic disorder: possible evidence that some anticonvulsants disturb imprinted genes?** *J.C.S. Dean[1], J.H.G. Williams[2], A.D. Rasalam[2], S.J. Moore[3], H. Hailey[1], D.J. Lloyd[1], S. Yousaf[1], P.D. Turnpenny[4]*. 1) Dept Medical Genetics, Medical Sch, Aberdeen, Scotland; 2) Dept Child Health, University of Aberdeen, Scotland; 3) Memorial University of Newfoundland, St John's, Newfoundland; 4) Royal Devon and Exeter Hospital, Exeter, England.

Autistic disorders have a prevalence of approximately 2/1000 and affect boys 3.8 times more frequently than girls. In our study of 255 Aberdeen children born to mothers taking anticonvulsants during pregnancy, case record review found that 12 children reported by parents to have an autistic or semantic-pragmatic communication disorder fulfilled the DSM-IV criteria for a diagnosis of autism (11) or Asperger syndrome (1). These children also had other clinical features suggestive of a fetal anticonvulsant syndrome, and were exposed to valproate and carbamazepine, either singly or in combination. The sex ratio was 1:1. In one male case, there was a family history of autism. Overall, the prevalence of autism in this group is very high (47/1000), and a large proportion of this can be attributed to the increased prevalence in girls (12 times the expected level), the prevalence in boys is only 3 times greater than expected. Previous studies have shown that in girls with Turner syndrome and autism, the functional X chromosome is maternal in origin, and it has been suggested that imprinted X-linked genes may contribute to the male preponderance in autism. The relatively high frequency of autism in girls with fetal anticonvulsant syndrome group, taken with the apparent interactions of valproic acid with methylation pathways (MTHFR) and other transcriptional regulators, is compatible with the hypothesis that some anticonvulsant drugs may be teratogenic because of effects on imprinting or related transcriptional control mechanisms in the developing embryo.

Program Nr: 32 from 2002 ASHG Annual Meeting

**Mortality in trisomy 13 and trisomy 18: An update and evaluation of factors associated with longer survival.** *S.A. Rasmussen[1], L.Y. Wong[2], Q.H. Yang[1], K. May[3], J.M. Friedman[4].* 1) CDC, Atlanta, GA; 2) ATSDR, Atlanta, GA; 3) Emory University School of Medicine, Atlanta, GA; 4) University of British Columbia, Vancouver, Canada.

Although trisomy 13 and trisomy 18 are generally considered to be lethal, long-term survival of patients has been reported. We sought to evaluate mortality in persons with trisomy 13 or 18 using two population-based strategies. In the first, infants with trisomy 13 or 18 born during 1968-1999 were identified using the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based birth defects surveillance system. Dates of death were documented using hospital records, Georgia vital records, and the National Death Index. In the second analysis, we used the Multiple-Cause Mortality Files (MCMF), compiled from U.S. death certificates, from 1979 through 1997. Using MACDP, we identified 70 liveborn infants with trisomy 13 and 114 liveborn infants with trisomy 18. Median survival time was 7 days (95% confidence interval [CI]: 3-15) for persons with trisomy 13 and 14.5 days (95% CI: 8-28) for persons with trisomy 18. For each condition, 91% of infants died within the first year. Neither race nor gender affected survival for trisomy 13, but for trisomy 18, females and infants of races other than white appeared to survive longer. The presence of a heart defect did not appear to affect survival for either condition. Using MCMF, we identified 5,514 persons with trisomy 13 and 8,750 persons with trisomy 18 listed on their death certificate. Median ages at death for persons with trisomy 13 and trisomy 18 were both 10 days. 5.61% of persons with trisomy 13 and 5.57% of persons with trisomy 18 died at age 1 year or greater. Race and gender appeared to affect survival in both conditions, with females and blacks showing higher median ages at death. Although survival is greatly impacted by trisomy 13 and trisomy 18, 5-10% of persons with these conditions survive beyond the first year of life. These population-based data are useful to clinicians caring for patients with these trisomies or counseling families with infants or fetuses diagnosed with trisomy 13 or 18.

Program Nr: 33 from 2002 ASHG Annual Meeting

**Turner Syndrome: Adaptation Across the Lifespan.** *B.B. Biesecker, A.M. McInerney-Leo.* Medical Genetics Branch, NHGRI/NIH, Bethesda, MD.

Turner syndrome affects girls and women medically, psychologically and socially across the lifespan. Prior research has focused on the psychological impact of living with short stature and infertility. Yet, few studies have captured the narratives of affected individuals about what has been most useful in helping them to adapt to their condition. We undertook a qualitative interview study of 64 individuals, 54 affected girls and women (ranging in age from 10-59 years with an average age of 30 years) and 10 parents of affected girls. The age at diagnosis ranged from the neonatal period to age 35 years. The focus of the study was on family relationships and communication, acceptance and nurturing, and social interactions and stigma. Girls and women were asked what health care providers can do to enhance acceptance and adaptation to their condition. Participants in the study described multiple ways that their families dealt with the diagnosis ranging from secret-keeping to parents disclosing all information to affected daughters. Participants often described experiencing teasing and social isolation as children. Some families provided strategies to resist the hurt that can stem from such treatment. Many of the participants found validation and support through meeting other girls or women with Turner syndrome. Many participants have also successfully identified health care providers who they perceive to be open and receptive to their emotional lives as well as their medical needs. Suggestions given by participants to hypothetical parents of a newborn with Turner syndrome were to treat their daughter "normally" minimizing the condition and focusing on what was unique and interesting about her as a girl. Participants suggested providing sufficient accommodation and medical care to promote independence. Most of the women and girls with Turner syndrome asked not to be stereotyped but rather seen as individuals, while others identified strongly with the label of Turner syndrome and found solidarity and understanding from being viewed as affected with the condition. We present our interpretation of the different roles the diagnosis of Turner syndrome plays in the adaptation of these girls and women.

Program Nr: 34 from 2002 ASHG Annual Meeting

**Detection of occult Y mosaicism in 45, X Turner Syndrome: Quantitation of Y chromosome material by real time PCR.** *S.M. Zeng[1], A.L. Reiss[2], J. Yankowitz[1].* 1) Dept of OB/GYN, Univ Iowa Hosps & Clinics, Iowa City, IA; 2) Dept of Psychiatry and Behavioral Sciences, Stanford Univ School of Medicine, Stanford, CA.

Turner syndrome (TS) is one of the most common cytogenetic abnormalities in humans. Occult mosaicism has been given special attention because TS patients with Y chromosome material have a risk as high as 30% for development of gonadoblastoma. The rate of occult Y mosaicism varies from 0-61% in the literature due to methodological differences. Our aim is to search for hidden mosaicism by conventional PCR, nested PCR, and to quantify occult Y chromosome material by quantitative real time PCR in 45,X TS patients.

Forty-four TS patients cytogenetically confirmed as 45,X, without second cell lines, were chosen for this investigation. Genomic DNA was extracted from the peripheral blood of the patients. To identify cryptic Y material three loci (SRY, DYZ3 and DYZ1) on different Y regions were amplified by conventional PCR. Additionally, two primers were used for nested PCR to examine AMXY locus. Two TaqMan$^{TM}$ probes and two sets of primers, one for SRY and another for a reference gene (b actin), were selected for quantitative real time PCR. Real time PCR was performed in PE Applied Biosystems 7900TH sequence detection system. The copy number of SRY and b-actin was automatically evaluated from their standard curves by the 7900TH sequence detection software. Conventional and nested PCR analysis revealed no Y chromosome material in all 44 TS patients. Real time PCR found that 8.6% TS cases (3/35) that adequately amplified were positive for Y-material and the rate of Y material cell is very low, ranging from 1/237-1/361 in peripheral blood of these 3 45.X TS patients.

Our results indicated that real time PCR is a sensitive method for detection of occult Y material. This method can find an extremely low rate of Y mosaicism and quantitate the copy number of Y chromosome material. 8.6% of TS patients were positive for Y chromosome material using real time PCR. The clinical significance of this finding needs further studies.

Program Nr: 35 from 2002 ASHG Annual Meeting

**Mapping without metaphases: haploinsufficiency for SOX2 associated with an apparently balanced 3q26.3 breakpoint in a child with bilateral anophthalmia.** *J. Fantes[1], N. McGill[1], P.N. Howard-Peebles[2], V. van Heyningen[1], D. FitzPatrick[1]*. 1) MRC Human Genetics Unit, Edinburgh, UK; 2) Genetics & IVF Institute, Fairfax, VA.

Several independent structural chromosome anomalies involving 3q27 have been associated with severe bilateral anophthalmia. We report characterisation of the 3q breakpoint in a child with bilateral anophthalmia associated with a de novo, apparently balanced translocation t(3;11)(q26.3;p11.2) [Driggers R.W. et al (1999) Am J Med Genet 87:201-202]. The lack of metaphases in the limited sample available precluded conventional metaphase FISH so interphase nuclei were analysed instead. Using a 3q chromosome paint it was possible to unambiguously map locus specific FISH probes to one of the three possible 3q chromosomal compartments: normal 3q, der(3), der(11). With this approach we identified both the translocation breakpoint and a ~740 kb deletion just proximal to the breakpoint. The human genome draft of a 1.6 Mb region covering the breakpoint and deletion showed three gaps in the sequence contig, as well as one known and several predicted genes. We have shown that SOX2, a gene expressed in the developing optic placode, is located in one of these gaps and is deleted in this patient. Two other cases with an anophthalmia/microphthalmia phenotype have visible deletions of 3q [Male A. et al (2001) J Med Genet 38: S30] and are also deleted for SOX2. We hypothesize that haplo-insufficiency of SOX2 leads to the anophthalmia/microphthalmia phenotype in all three cases. A further 23 unrelated cases of bilateral microphthalmia associated with a normal karyotype were screened for deletions in the SOX2 region but no abnormality was detected.

Program Nr: 36 from 2002 ASHG Annual Meeting

**Developing the GEM database (Genetics Education Materials database).** *K. Silvey[1], J. Livingston[2], P. Boyd[1], B. Therrell[2], M. Mann[3].* 1) Oregon Health & Sciences University, Portland OR; 2) University of Texas Health Science Center at San Antonio, San Antonio, TX; 3) HRSA-MCHB, Rockville, MD.

Genetics specialists, public health professionals, and policy makers are working to integrate genetics into public health programs. Because the various professionals working in public health genetics do not yet share a common vocabulary, finding resources can be time-consuming and frustrating. Commonly used Internet search engines often generate overwhelming numbers of documents or incomplete results. The GEM database provides easy access to policy documents and educational materials. It is a project of the National Newborn Screening and Genetics Resource Center (NNSGRC), a cooperative agreement between The University of Texas Health Science Center at San Antonio and HRSA/Maternal and Child Health Bureau. It is accessible through the NNSGRC web site, http://genes-r-us.uthscsa.edu. The original GEM database was developed prior to widespread use of the Internet to catalogue education materials produced by the ten HRSA-funded regional genetics networks. A formative evaluation process is now being used to guide its enhancement. Representatives from national public health and genetics professional organizations were surveyed to help prioritize potential search features and publications to be added. Ease of use and direct links to on-line documents were high priorities. The search options were modified and now include condition, distributor, format, language, and 22 subjects. Approximately 250 policy statements, guidelines, and other resources were added to the 150 original listings. Likely users were then asked to evaluate ease of navigation, clarity of instructions, and overall utility. Earlier this year, state genetics coordinators were asked what materials they need to access and what materials they are willing to share. The next step is to add state-specific documents. The accuracy of listings is ensured by monthly electronic verification of Internet links and annual review of entered information by project staff and document distributors. The usability of the database will be evaluated by tracking the search options selected and through annual user surveys.

Program Nr: 37 from 2002 ASHG Annual Meeting

**Awareness of Genetic Testing for Increased Cancer Risk in the Year 2000 National Health Interview Survey.** *S. Thomas[1,2], L. Wideroff[1], N. Breen[1], R. Croyle[1], A. Freedman[1].* 1) Division of Cancer Control and Population Sciences, NCI, Bethesda, MD; 2) Cancer Prevention Fellowship Program, NCI, Bethesda, MD.

Although genetic tests for inherited cancer susceptibility (GTICS) have primarily been used in a research setting, they are gradually being utilized in mainstream clinical practice as protocols for clinical use evolve. The purpose of this study was to estimate the percentage of the U.S. adult population who have heard of GTICS, and assess whether the percentage varies by demographic, family history, behavioral, and health care use factors. The National Health Interview Survey is a nationally representative survey that uses an in-person, computer-assisted household interview. The statistical analysis included 27,211 men and women, ages 25 and older, who responded yes or no to having heard of GTICS. Weighted percentages (standard errors) were calculated, and test awareness was modeled in multivariate logistic regression analyses, adjusting for demographic, family history, behavioral, and health care factors. Based on self-reports, 43.7%(±0.4) of the U.S. adult population has heard of GTICS. Percentages were highest in people with post-graduate education (68.4%; ±1.1) and lowest in those with <12th grade education (18.3%;±.76). Percentages were highest among Whites (50.2%; ±0.5), intermediate among African-Americans (33.1%;±1.0), Asian/Pacific Islanders (28.8%; 2.1), and American Indian/Alaskan Natives (32.3%; ±4.8), and lowest among Hispanics (20.8%; ±0.8). After adjustment for covariates, higher GTICS awareness was associated with being 40-59 years old, White, female, a resident of the Northeast, physically active, and having higher education and income, high self-perceived cancer risk, health insurance coverage, recent contact (<1 year) with a health professional, and parental history of cancer. There was no association with having a usual place to go when sick or with a personal history of cancer. Though GTICS is not recommended for the general population, these results provide insight into strategies for targeting efforts to reach a more diverse group of high risk populations that may benefit from genetic counseling and testing.

Program Nr: 38 from 2002 ASHG Annual Meeting

**Genetics in Ontario: Mapping the future.** *A.M. Summers*[1,2]. 1) Genetics Program, North York General Hosp, Toronto, ON, Canada; 2) Chair, Ontario Provincial Advisory Committee on New Predictive Genetic Technologies.

Ontario is one of the largest provinces in Canada with a population of ~11 million. As in many publicly funded jurisdictions, the government has become concerned about the potential pressures on the health care system resulting from the advent of predictive and predisposition testing for common adult-onset disorders. In April 2000, the Minister of Health and Long Term Care announced that there would be a Provincial Advisory Committee on New Predictive Genetic Technologies (PACNPGT). The mandate of this committee would be to develop principles, guidelines and broad criteria to guide operational decision-making regarding the introduction of new genetic technologies by the Ministry of Health and Long Term Care (MOHLTC). The work of the committee was handled through six subcommittees (ethical/legal, evaluation, laboratory, clinical, psychosocial and education) which were designated both general and specific tasks. Each subcommittee was asked to produce a written report within a year. In addition to the subcommittee work, the PACNPGT held a horizon scanning day where a number of experts were invited to discuss their views on the future of genetic medicine. The MOHLTC also funded a public poll on a variety of attitudes of Ontario citizens to genetics in medicine. All of this data was combined by the PACNPGT into a final report which included 13 recommendations. The PACNPGT recommended that there be an ongoing multidisciplinary committee to oversee the process; that all genetic technologies be considered as part of a health care programme; that each technology be evaluated on a scientific, epidemiological, ethical, legal, psychosocial and clinical basis; that education programmes be undertaken for health care providers, teachers and the public; that quality be assured for all aspects of the programme; that human resources be increased; and, that ethical issues such as discrimination, research ethics, informed consent, duty to warn and testing of minors be fully examined and where appropriate, regulation or legislation be put into place. These recommendations will be discussed.

Program Nr: 39 from 2002 ASHG Annual Meeting

**Impact of Genetic Discrimination on BRCA testing and Medical Management Decisions.** *M. Merrill[1,2], R. Nagy[1,2], S. Zyzansky[1], B. Lamb[1], G.L. Wiesner[1,2], A. Matthews[1,2].* 1) Case Western Reserve University, Cleveland, OH; 2) University Hospitals of Cleveland, Center for Human Genetics, Cleveland, OH.

Genetic testing for common adult-onset conditions has resulted in concerns regarding genetic discrimination among patients, health care professionals, and the media, and these concerns may be a barrier to genetic testing. As a result, this study examined the impact of worry about genetic discrimination on BRCA1/2 testing uptake and medical management decisions among 137 women using a mailed questionnaire. Responses to seven questions on the survey were used to develop a discrimination scale to quantify each respondent's overall level of concern regarding genetic discrimination. This scale has both high internal consistency (a=0.9381) and external validity. Participants with high discrimination scale scores, and thus high concerns about genetic discrimination were more likely to decline BRCA testing (p=0.03). Because concern about discrimination was associated with BRCA testing decisions, factors influencing the uptake of testing were examined. Those undergoing BRCA testing were more likely to be Ashkenazi Jewish (p=0.006), have a personal history of cancer (p=0.001), and have an insurance policy that was not individually rated (p=0.013). Despite the fact that concern for genetic discrimination predicted BRCA testing decisions, this study was unable to document any instances of genetic discriminaiton. A small percentage of respondents reported difficulties with insurance providers unrelated to genetic test results. Furthermore, high concern for genetic discrimination did not predict use of cancer screening, prophylactic surgery, or chemoprevention to lower cancer risk. Finally, the study was unable to document evidence of adverse selection. This study confirms that patient perception is discrepant with actual occurrence of genetic discrimination. Although it appears that patient perception of genetic discrimination is influencing decision making for BRCA testing, it is encouraging that this concern did not prevent high risk individuals from seeking out options for cancer risk reduction or screening.

Program Nr: 40 from 2002 ASHG Annual Meeting

**“Bringing you the world of genetics”: Direct-to-consumer genetic services on the internet.** *S.E Gollust, S.C. Hull, B.S. Wilfond.* Bioethics Research Section, NIH/NHGRI/MGB, Bethesda, MD.

Consumers are using the Internet with increasing frequency to obtain medical information and to order clinical tests and medications without a physician’s prescription. To assess the availability of genetic services on the Internet, we conducted a systematic search in May 2002 to identify sites that allow consumers to obtain genetic services directly. We identified 105 sites that offered direct-to-consumer services, including 88 within the US and 17 internationally. A majority of the sites (73%) allowed at-home sample collection. The sites primarily offered non-health related services, and many sites offered more than one service, including paternity testing (83%), identity/forensic testing (56%), and DNA banking (24%). Clinical genetic tests were offered in 14 sites (13%), including 5 sites that also offered non-health related services. Of these 14 clinical sites, 4 asked consumers to contact the company for ordering information, 4 allowed consumers to order tests but required a physician to receive results, and 6 allowed consumers both to order and receive test results directly. The tests offered on these 6 sites ranged from standard tests to more novel applications, including: hereditary hemochromatosis; alpha-1 antitrypsin deficiency; a pharmacogenetic test for cytochrome p450 alleles; testing to provide dietary and lifestyle advice based on a profile of polymorphisms including MTHFR, NAT2, MnSOD, and ALDH2; testing for risk of alcohol and drug addiction based on dopaminergic alleles; and a test (and remedy) for age-related DNA damage. Of these 6 sites, 3 described risks associated with genetic testing, including emotional consequences, genetic discrimination, and health risks related to making medical or behavioral changes, and none offered genetic counseling services. Sites that offer clinical genetic tests directly to consumers and bypass providers, particularly when risks are not adequately disclosed and counseling not provided, may expose consumers to unnecessary risks. If the availability of clinical testing on the Internet continues to expand in an unregulated environment, there is greater potential for the public to be harmed.

Program Nr: 41 from 2002 ASHG Annual Meeting

**Patient reports of topics discussed in genetic counseling: is the psychosocial dimension missing?** *D.C. Wertz.* Soc Sci, Ethics/Law,ShriverDiv, Univ Massachusetts Med Sch, Waltham, MA.

To investigate the content of counseling, we surveyed 710 patients or parents before their first counseling sessions at 12 general genetics clinics in the US and Canada, using anonymous questionnaires. 476 (67%) responded. Of these, 310 (65%) returned post-counseling questionnaires. Respondents were mostly women (91%), white (89%), working class (70%), with a median 13 years education and median family income $25,000-45,000. They came to counseling for information about their child (38%), fetus (21%), whether to have a child (14%), PND for advanced maternal age (11%), or adult conditions (16%). 2-4% reported that the following topics were discussed in depth: costs of caring for someone with a genetic condition; resources available; joining a support group; psychological counseling; effects of caring for someone with a genetic condition on the marriage, on the family's other children; on family life; or on society; providing care at home; group home or special residence; hospitalization; telling an employer or insurer about a genetic diagnosis. An additional 4-8% reported that these topics were just mentioned. Telling a partner about a genetic diagnosis was discussed in depth in 19% of sessions; telling blood relatives in 12%; what a child with a genetic condition may be like as an adult in 15%, with an additional 15-24% reporting these topics as just mentioned. The only topic discussed in depth in a majority of sessions (63%) was why a genetic disorder occurs. However, many respondents thought that a genetic condition would have a moderate to extremely serious effect on family life (55%), financial situation (54%), relationship with spouse/partner (42%), own quality of life (42%), other children (42%), and relationship between home and job (35%). Most (86%) reported satisfaction with counseling, even though these topics were not discussed in depth. Results suggest that although patients may not expect genetics professionals to discuss psychosocial issues, there is considerable need for such for such discussions.

Program Nr: 42 from 2002 ASHG Annual Meeting

**Comparative willingness to consider genetic testing for colorectal cancer in Caucasian- and African-Americans.**

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The attitudes of African-Americans toward genetic testing for colorectal cancer are not well documented. We conducted a mail survey of the first degree relatives (FDRs) of colorectal cancer (CRC) patients in families without an autosomal dominant CRC syndrome. Respondents completed questions pertaining to demographic characteristics, cancer screening behavior, attitudes about cancer and cancer screening, attitudes about genetic testing for CRC, and perceived risk for CRC. Forty-one African-Americans (31 women, 10 men) completed surveys, reflecting significantly greater participation among African-American women ($c^2 = 21.51$, $p = 0.0001$). Each of them was matched on sex, age, and education with a Caucasian survey responder. Mean age was 51; about 50% of both groups had a college degree. Marital and employment status were equivalent in the African-American and Caucasian-American groups (56%, 76% married; 61%, 56% employed full time). There were no significant group differences on cancer screening behavior, attitudes about cancer screening, attitudes about genetic testing for CRC, or perceived risk for CRC. In both groups: about 60% had endoscopic screening at least once; 88% would be screened with a physician's recommendation; about 20% would avoid screening due to fear; about 50% somewhat- or strongly-agreed that CRC is preventable; about 90% were likely to take a gene test for CRC; and about 80% believed their risk for CRC to be a little or much higher than chance. However, African-Americans perceived themselves as more likely to get CRC compared to other racial groups ($p=0.002$) and had stronger expectations that genetic information would be used to discriminate against people of color ($p=0.0001$). We conclude that hypothetical willingness to undertake genetic testing for CRC is virtually identical among Caucasian- and African-Americans, when controlling for age, sex, and education. Because African-Americans are more likely to expect that genetic information will be used against people of color, actual uptake of testing may be lower than among Caucasians.

Program Nr: 43 from 2002 ASHG Annual Meeting

**Hearing Status Influences Attitudes Toward Genetic Testing for Deafness.** *A. Martinez[1], J. Linden[1], L.A. Schimmenti[2], C.G.S. Palmer[2]*. 1) Cal State Univ Northridge, CA; 2) University of California, Los Angeles, CA.

Genetic testing for mutations in Connexin 26 has been proposed to improve the detection of deaf and hard-of-hearing newborns. Due to different medical and cultural perspectives toward deafness, the attitudes and concerns of hearing, deaf and hard-of-hearing individuals must be assessed prior to the implementation of this test. Objective: To compare the attitudes, knowledge and concerns of hearing, deaf/hard-of-hearing individuals toward genetic testing for deafness in a non-medical and non-culturally influenced setting in the U.S. Methods: A self administered questionnaire was completed by hearing (n = 133), deaf (n = 60) and hard-of-hearing (n = 29) college students in the US. Knowledge and attitudes toward genetic counseling and genetic testing for deafness were assessed. Participants were classified as hearing(h) or deaf/hard-of-hearing (hoh), the responses of the two groups were compared. Results: Significant differences between the two groups were found. The majority of the h group held positive attitudes toward genetic testing for deafness. In contrast, the d/hoh group indicated greater ambivalence, but no clear rejection of such testing. Both groups were more likely to favor newborn genetic testing for deafness compared to prenatal testing. While d/hoh participants indicated a mean perceived chance to have a deaf child more than double (30.46%) that of the h participants (12.81%), the h participants indicated more interest in genetic counseling. The majority of the h participants (73%) indicated a preference to have a hearing child, while the majority of the d/hoh participants (81%) indicated no preference. 7% of the deaf participants indicated a preference for a deaf child. The two groups were similar in terms of lack of sophistication about genetic testing and genetic counseling, and concern about insurance discrimination. In general, age, ethnicity, and cultural identification did not have a significant effect on the results. Conclusion: differences in hearing status may influence the acceptance of genetic testing for deafness. Introducing newborn genetic testing for deafness may be an acceptable adjunct to newborn hearing screening.

Program Nr: 44 from 2002 ASHG Annual Meeting

**Determining the genetic basis of pseudoxanthoma elasticum (PXE): A consortium driven research network partnering an international non-profit patient advocacy organization, academic institutions, and a biotechnology corporation.** *P.F. Terry[1], F. Frueh[2], N. Taylor[2], J. Breen[2], M.A. Marino[2]*. 1) PXE International, Inc, Sharon, MA; 2) Transgenomic, Inc, Gaithersburg, MD.

An innovative program to characterize genetic alterations that result in the inherited disorder PXE was developed between PXE International, academic institutions, and the Transgenomic Corporation. The ultimate goal of the consortium is to develop and implement a standardized mutation detection assay for the ABCC6/MRP6 gene. This effort includes the global coordination of patient sample acquisition, the development of a sensitive screening protocol, and the implementation of genotyping assays. A comprehensive mutation detection assay for the ABCC6/MRP6 gene will provide important information for PXE patients and family members, contribute towards the understanding of the genotype/phenotype relationships for this disorder, and indicate important functional domains in the ABCC6/MRP6 membrane transport protein. An integral part of this research network is the management and dissemination of data collected from laboratories throughout the world. The success of this program requires a high level of commitment and a well-coordinated effort between industry, academic, and patient advocacy group members. This program will directly benefit PXE patients and family members, and will serve as a model of collaborative efforts and global coordination for other rare genetic diseases.

Program Nr: 45 from 2002 ASHG Annual Meeting

**The pattern of inheritance of X-linked traits: not dominant, not recessive, just X-linked.** *W.B. Dobyns[1], A. Filauro[2], A.S. Chan[2], A. Ho[2], N.T. Ting[2], C. Ober[1,2].* 1) Dept Human Genet, Univ of Chicago, Chicago, IL; 2) The College, Univ of Chicago, Chicago, IL.

The existence of an X-linked disease, hemophilia, was described in the Talmud in the 1st centuries A.D. The terms "dominant" and "recessive" were first used by Mendel for autosomal traits, and were applied to X-linked inheritance by Morgan in 1915. Human genetics texts have since used the terms X-linked dominant (XLD) and XL recessive (XLR). XLD has been defined as vertical transmission in which daughters of affected males are always affected and transmit the disorder to offspring of both sexes. XLR has generally been defined as vertical transmission in which unaffected "carrier" females pass the disease to affected sons (Emery and Rimoin, 1997). Most texts suggest that XLR inheritance is common while XLD inheritance is rare. However, many X-linked disorders do not fit these rules. We reviewed the literature for 38 X-linked disorders and recorded information on penetrance and expressivity in both sexes. We defined penetrance as the presence of any clinical disease, but not abnormal blood test results. We defined expressivity as the percent of affected individuals with the severe phenotype. Among the 38 diseases, penetrance in males was >90% in 35/38 (92%) diseases and intermediate (10-90%) in 3 (8%). Expressivity was high in males with >50% of affecteds having a severe phenotype in 31/38 (82%) of diseases. Penetrance in females was 90% or higher in 12/38 (32%), intermediate (10-90%) in 11/38 (29%), and low (<10%) in 15/38 (39%) diseases. Expressivity was unexpectedly high in females, with >50% of affecteds having a severe phenotype in 8/38 (21%). We conclude that the terms XLD and XLR are difficult to define and impractical in common use. They do not capture the extraordinarily variable expressivity of X-linked diseases or take into account the multiple mechanisms that can result in disease expression in females, which include cell autonomous expression, skewed X inactivation, clonal expansion and somatic mosaicism. We recommend that usage of the terms XLD and XLR be discontinued, and that all such disorders be simply described as having "X-linked" inheritance.

Program Nr: 46 from 2002 ASHG Annual Meeting

**Identification of the gene responsible for the cblA complementation group of disorders of human vitamin $B_{12}$ metabolism based on analysis of prokaryotic gene arrangements.** *C.M. Dobson[1], T. Wai[2], D. Leclerc[2], A. Wilson[1], X. Wu[1], C. Doré[2], T.J. Hudson[2], D.S. Rosenblatt[2], R.A. Gravel[1]*. 1) University of Calgary, Calgary, AB, Canada; 2) McGill University Health Center, Montreal, QB, Canada.

Vitamin B12 (cobalamin, Cbl) is an essential cofactor of two enzymes, methionine synthase and methylmalonyl-CoA mutase (MCM), with methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), as the cofactor forms, respectively. The conversion of the vitamin to its coenzyme forms requires a series of biochemical modifications for which several genetic disease are known, comprising eight complementation groups, cblA through cblH. The objective of this study was to clone the gene responsible for the cblA complementation group, thought to represent a mitochondrial Cbl reductase in the pathway leading to AdoCbl synthesis. Through a bioinformatics approach, examining genes in close proximity to the gene for MCM in various bacteria and searching for orthologous sequences in the human genome identified potential candidates. A cblA candidate gene was assembled and evaluated for deleterious mutations in cblA patient cell lines. A screen of 15 patient cell lines by heteroduplex analysis yielded a homolallelic 4 bp deletion in one cell line that would result in a truncated protein and two additional cell lines heteroallelic for the deletion, one of which also has an 8 bp deletion. Other identified mutations include nucleotide substitutions that would result in a stop codon and in amino acid substitutions. These data confirm that the identified gene corresponds to the cblA complementation group. Interestingly, it appears to code for a protein that is unrelated to the anticipated Cbl reductase. Instead, its deduced amino acid sequence reveals a domain structure which belongs to the AAA ATPase superfamily that encompass a wide variety of proteins including ABC transporters and accessory proteins binding both ATP and GTP. We speculate that we have identified a component of a new transporter or accessory protein that is involved in the translocation of vitamin B12 into mitochondria. This study demonstrates the utility of "gene finding" using bacterial operons to identify genes belonging to the same metabolic pathway.

Program Nr: 47 from 2002 ASHG Annual Meeting

**Hereditary Inclusion Body Myopathy due to mutations in the gene for UDP-GlcNAc 2-epimerase/ManNAc kinase.** *M. Huizing[1], C.R. Hermos[1], O.M. Vasconselos[2], E. Orvisky[3], D. Krasnewich[4], M.C. Dalakas[2], W.A. Gahl[1]*. 1) NICHD; 2) NINDS; 3) NIMH; 4) NHGRI, NIH, Bethesda, MD.

Patients with hereditary inclusion body myopathy (HIBM) exhibit quadriceps-sparing limb weakness of adult onset, with rimmed vacuoles and filamentous inclusions in muscle tissue. Linkage analysis of a Middle Eastern Jewish isolate allowed identification of the HIBM-causing gene, i.e., UDP-N-acetylglucosamine (UDP-GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase, or *GNE*. The GNE enzyme is bifunctional and rate-limiting in sialic acid biosynthesis. Mutations in its allosteric site cause overproduction of free sialic acid in the dominant disorder sialuria, clinically characterized by developmental delay, coarse facies, and hepatomegaly. In a non-Jewish American HIBM patient, we found 2 compound heterozygous *GNE* mutations, one in the kinase and one in the epimerase domain. We assayed GNE epimerase activity using UDP[3H]GlcNAc as substrate and measuring the product, [3H]ManNAc. The patient's activity was only 10% of normal in cultured muscle cells, but 30% of normal in cultured fibroblasts. Tissue specific residual activity might be an explanation for this discrepancy. Sialic acid modifications of cell surface glycoproteins are crucial for cell adhesion and signal transduction. Our HIBM patient's fibroblasts showed defective glycoconjugate sialylation as follows: 1. Immunoblots stained with anti-LAMP2, a highly glycosylated membrane protein, showed a broad 'smear' for the patient compared with a single band for controls, reflecting glycoconjugates of different sizes on the patient's glycoproteins. 2. Using the lectin WGA-FITC, which recognizes sialic acid end-groups on glycoproteins, immunofluorescence showed a punctated pattern throughout normal fibroblasts, but only residual staining around the Golgi in HIBM fibroblasts. 3. When growing the patient's cells in media containing free sialic acid or ManNAc, both the LAMP2 staining and the WGA fluorescence were corrected to a normal pattern. We conclude that mutations in GNE can cause two clinically distinct diseases, and that supplementation with ManNAc or with free sialic acid may provide therapy for patients with HIBM.

Program Nr: 48 from 2002 ASHG Annual Meeting

**Lathosterolosis, a novel defect of cholesterol biosynthesis in humans associated with multiple congenital malformations and mental retardation.** *N. Brunetti-Pierri[1], G. Corso[2], M. Rossi[1], I. Annunziata[3], A. Battagliese[1], P. Ferrari[4], A. Ballabio[3], A. Dello Russo[2], G. Andria[1], G. Parenti[1]*. 1) Dept Pediatrics, Federico II Univ, Naples, Italy; 2) Dept Biochemistry and Medical Biotechnologies, Federico II Univ, Naples, Italy; 3) Telethon Institute of Genetics and Medicine, Naples; 4) Dept. Pediatrics, Univ. Modena, Italy.

Inborn errors of cholesterol biosynthesis have recently emerged as a cause of human malformation syndromes. The best studied example of this group of disorders is the Smith-Lemli-Opitz (SLO) syndrome, due to 7-dehydrocholesterol reductase deficiency. We have identified a new defect of cholesterol biosynthesis in a female patient affected by severe mental retardation, multiple congenital anomalies, including cranio-facial dysmorphism, polydactyly, vertebral anomalies, and liver disease. In this patient plasma and cell sterol profiles by GC-MS showed increased lathosterol, suggesting a metabolic block in the conversion of lathosterol into 7-dehydrocholesterol. The enzyme involved in this reaction is sterol C-5-desaturase (SC5D). The activity of this enzyme was defective in the patients fibroblasts. Patients fibroblasts cultured in the presence of 3H-mevalonate, an early intermediate of cholesterol biosynthesis, showed accumulation of 3H-lathosterol, while in controls 3H-cholesterol was normally synthesized. Sequence analysis of SC5D gene in the patients DNA showed the presence of two missense mutations (R29Q and G211D), confirming that the patient is affected by a defect of SC5D. The clinical characterization of a new defect of cholesterol biosynthesis provides additional indications for plasma and cell sterol profiling in patients with unexplained malformations and mental retardation and further confirms that defects in the post-squalene steps of this metabolic pathway are associated with congenital malformations.

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Program Nr: 49 from 2002 ASHG Annual Meeting

**Biochemical and molecular diagnosis of an X-linked lethal form of neonatal cholestasis named CADDS.** *S.J. Steinberg[1], A. Patel[1], K. Johnson[1], G.V. Raymond[1], G.R. Cutting[2], A.B. Moser[1], P.W. Watkins[1], K.D. Smith[1], H.W. Moser[1]*. 1) Kennedy Krieger Institute; 2) Institute of Genetic Medicine, JHU, Baltimore, MD.

Neonatal cholestatic liver disease occurs in about 1 in 10,000 live births. Newborn males diagnosed with the newly described disorder CADDS (Contiguous *ABCD1 DXS1357E* Deletion Syndrome) present in the neonatal period with hypotonia and cholestatic liver disease (Corzo et al, 2002; AJHG 70:1520-1531). There is some overlap in clinical and biochemical phenotype between CADDS and peroxisomal biogenesis disorders such as Zellweger syndrome. However, CADDS patients do not have craniofacial dysmorphism, a feature that is commonly associated with most disorders presenting in the neonatal period with a defect in peroxisomal fatty acid metabolism. Thus, clinicians may feel disinclined to measure plasma very long chain fatty acids (VLCFA) in babies at risk for CADDS. We have recently diagnosed 5 patients with CADDS, 3 of whom were identified in the past 10 months. All five neonates died at less than 12 months of age. Patients were diagnosed based upon these criteria: (1) increased plasma very long chain fatty acid levels; (2) absence of ALD protein in the peroxisomes of cultured fibroblasts; (3) presence of a large 5-deletion of ABCD1; and, (4) extension of the Xq28 deletion into the neighboring gene *DXS1357E*. The extent of each deletion is variable and so far unique to each patient. We identified the breakpoints of the smallest deletion. This deletion spans 33kb and eliminates the 5-ends and shared promoter region of *DXS1357E* and *ABCD1* due to their head-to-head orientation. We have designed a probe for analysis of this critical region by FISH and currently we are testing its utility in the rapid diagnosis of probands and carrier detection of their mothers. We recommend that neonates, especially boys, with cholestasis, hypotonia and developmental delay in the absence of craniofacial dysmorphism have plasma VLCFA measured. If increased, cells should be tested for the absence of ALDP immunocytochemically. Finally, DNA analysis in the region of *ABCD1* and *DXS1357E* should be performed to test for a deletion in the currently identified critical region.

Program Nr: 50 from 2002 ASHG Annual Meeting

**Creatine transporter defect: a newly recognized X-linked disorder.** *G. Salomons[1], S.J.M. van Dooren[1], N.M. Verhoeven[1], K.M. Cecil[2], W.S. Ball[2], G. Uziel[3], CH. Schwartz[4], T.J. de Grauw[5], C. Jakobs[1].* 1) Dept of Clinical Chemistry, VU Univ Med Center, Amsterdam, Netherlands; 2) Div of Radiology, Children's Hosp Med Center Cincinnati, OH; 3) Div of Neurology, Children's Hospital Medical Center Cincinnati, Cincinnati,OH; 4) Dept of Child Neurology Milan, Italy; 5) J.C. Self. Res. Inst., Greenwood, SC.

Introduction: We identified a new inborn error of metabolism caused by a defect in the X-linked creatine transporter/CRTR mapped at Xq28 (MIM 300036) . An X-linked creatine transporter was hypothesized because of 1) the absence of creatine in the brain of the index patient as indicated by H-MRS 2) the presence of marked creatine levels in urine and plasma ruling out a creatine biosynthesis defect 3) the absence of an improvement on creatine supplementation, and 4) the fact that the pedigree suggested an X-linked disease. The CRTR1 gene was mapped at Xq28. Methods: Creatine levels in body fluids and in cultured fibroblasts were determined by stable isotope-dilution GCMS. DNA sequence analysis was performed by standard molecular biology techniques using bigdye terminators and an ABI 310 machine. Results: Our hypothesis was proven by the presence of 4 different mutations in the CRTR1 gene (in five unrelated families) and by the impaired creatine uptake in fibroblasts of male patients. Three families were encountered in one institute (CHMCC) Conclusion: This newly discovered X-linked disorder might account for a considerable fraction of mental retardation observed in males. The expressive speech and language delay, autistic behavior, the absence of creatine in the H-MRS of the brain and the increased creatine levels in body fluids are hallmarks of this disorder. It might prove worthwhile to screen males with mental retardation in association with significant expressive speech and language delay or autism for creatine in urine.

Program Nr: 51 from 2002 ASHG Annual Meeting

**Urea Cycle Polymorphisms and Pulmonary Vascular Tone after Congenital Heart Surgery.** *F.E. Barr[1], H. Smith[1], K. Dyer[1], B. Christman[2], K. Christian[3], D. Drinkwater[3], M. Summar[1]*. 1) Department of Pediatrics; 2) Department of Medicine; 3) Department of Cardiothoracic Surgery, Vanderbilt University, Nashville, TN.

Background:Increased pulmonary vascular tone (PVT) can complicate the postoperative course of infants undergoing surgical correction of their congenital cardiac lesions. PVT is partially controlled by nitric oxide (NO). Arginine, the precursor to NO, is a product of the urea cycle (UC). We have identified a single nucleotide polymorphism (SNP)in the rate limiting UC enzyme, carbamyl phosphate synthetase I (CPSI). This SNP is a C to A transversion that results in a threonine to asparagine substitution at amino acid 1405 (T1405N), an important enzyme cofactor binding site. Three different genotypes exist at this SNP: CC, AC, and AA. Objective: To determine if T1405N genotype is a risk factor for increased post-op PVT after congenital heart surgery. Design/Method: Infants undergoing congenital cardiac surgery were studied. Parental informed consent was obtained. Increased PVT was defined as a mean PA pressure > 20mmHg or a clinical requirement for inhaled NO. Preop blood samples were obtained for T1405N genotype. Blood samples were obtained at 5 different time points for analysis of urea cycle intermediates including arginine. Results: 99 patients were enrolled. Surgical diagnoses included AVSD repair(15), VSD closure(19), Glenn shunt(27), Fontan procedure(6), Norwood stage 1(21), and Arterial Switch procedure(11). T1405N genotypes included CC (52/99, 53%), AC (35/99, 35%), and AA (12/99, 12%). Increased post-op PVT developed in 37/99 patients (37%). There was a significant effect of genotype on the development of increased postop PVT (21 CC, 14AC, 2 AA, p = 0.022). Patients who developed increased PVT had lower arginine levels at 24 and 48 hours postop. There was a significant effect of genotype on arginine levels at 48 hrs post-op (CC = 41, AC = 30, AA = 62 umol/l, p = 0.05). Conclusions: A genetic polymorphism in the rate limiting UC enzyme, CPSI, has an effect on postoperative arginine levels, the substrate for NO production, and subsequent development of increased postop PVT in infants undergoing congenital cardiac surgery.

Program Nr: 52 from 2002 ASHG Annual Meeting

**Abnormal GABA/Glutamine Metabolism in Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency, an Epilepsy Syndrome with Elevated CNS GABA.** *K.M. Gibson[1], M. Gupta[1], M. Callan[1], H. Senephansiri[1], M. Polinsky[1], M. Grompe[1], E.J. Novotny[2], P. Pearl[3], E.E.W. Jansen[4], A. Bakkali[4], C. Jakobs[4]*. 1) Molec & Med Genet, Oregon Hlth & Sci Univ, Portland, OR; 2) Ped Neurol, Yale Univ Sch Med, New Haven, CT; 3) Ped Neurol, Children's Natl Med Ctr, Washington, DC; 4) Ped. & Clin Chem, VU Med Ctr, Amsterdam, Netherlands.

Murine SSADH deficiency manifests neurologic impairment, increased GABA and gamma-hydroxybutyrate (GHB) and decreased glutamine in brain, and premature death from lethal seizures. Approximately 50% of human patients manifest either absence or tonic-clonic seizures. In the current study, we addressed the hypothesis that the GABA/glutamine axis in human patients would parallel abnormal findings in mice. Cerebrospinal fluid (CSF) from 13 patients (range 2-19 years; median 8) revealed increased GHB (449 +/- 41 (SEM) umol/L (range 203-703, n=13); control < 3), increased free GABA (0.37 +/- 0.11 umol/L (range 0.12-1.33, n=10); control < 0.17), increased total GABA (29.3 +/- 2.8 umol/L (range 13.7-40.5, n=11); control < 12.2) and decreased glutamine (337 +/- 21 umol/L (range 229-501, n=12); control 357-750). Magnetic resonance spectroscopy performed in an adult patient, with selective editing for GABA, revealed a GABA/total creatine ratio of 0.53 compared to a control value of 0.31 +/- 0.03, which was > 7 SD elevated. SSADH deficiency may represent the first pediatric/adult epilepsy syndrome with increased GABA/decreased glutamine. These metabolic disturbances likely disrupt the glial-neuronal GABA/glutamate neurotransmitter cycles, leading to aberrant glutamatergic excitatory and GABAergic inhibitory neurotransmission. Elevated GABA in early development may lead to altered neuronal migration, neurogenesis, myelination and synaptogenesis. The alterations in GABA are likely associated with the seizures observed in SSADH deficiency, but the mechanisms are unknown. Cross-sectional and longitudinal studies of murine and human SSADH deficiency will focus questions on mechanisms, and may provide insight into other epilepsy and neuropsychiatric disorders. Supported by NS 40270 and March of Dimes #1-FY00-352.

Program Nr: 53 from 2002 ASHG Annual Meeting

**Localization of holocarboxylase synthetase (HCS) in the nucleus of cells: A novel role for biotin in human biology?** *M.A. Narang, R. Dumas, L. Ayer, R. Gravel.* Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada.

Biotin-responsive multiple carboxylase deficiency (MCD) is an autosomal recessive disorder that is caused by mutations in holocarboxylase synthetase (HCS) resulting in early neurological, developmental and metabolic abnormalities. HCS catalyzes the ATP-dependent attachment of the vitamin biotin to biotin-dependent carboxylases making them functionally active. Using antibodies made to the expressed protein and to N- and C-terminal peptides of the protein, we found that the majority of HCS localizes to the nucleus rather than to the cytoplasm where carboxylases are found. Using digital deconvolution for maximizing spatial resolution, HCS was found distributed as distinct, speckle-like structures, evenly dispersed in the nucleus of interphase cells. By western blotting, *in situ* immunofluorescence and assay of biotin ligase activity, HCS was predominant in fractions containing chromatin proteins and the nuclear matrix. The addition of DNase I did not significantly alter the distribution of HCS suggesting that it is not tightly associated with chromatin. Significantly, HCS was induced to re-localize to the cytoplasm upon addition of ATP *in vitro* and *in vivo*, similar to other nuclear regulatory factors. Further characterization of HCS indicated that it is tyrosine phosphorylated, inhibition of which prevented re-localization of HCS to the cytoplasm in the presence of ATP. HCS was also observed in large complexes from early prophase to late telophase. It was not associated with condensed chromosomes. Instead, the large complexes containing HCS appeared to co-localize with lamin B, a component of the nuclear matrix that is dispersed due to nuclear membrane disassembly just prior to mitosis. Based on these data, we propose that in addition to its role in activation of carboxylases, HCS may be involved in chromatin organization, mitotic control or structural maintenance, associated with the nuclear matrix. These studies should have a profound impact on our understanding of the importance of biotin as a vitamin and biological molecule.

Program Nr: 54 from 2002 ASHG Annual Meeting

**Saposin's "Protector" Function for Their Cognate Enzymes.** *Y. Sun, G.A. Grabowski.* Dept Human Genetics, Children's Hosp Research Fndn, Cincinnati, OH.

Saposins (A, B, C and D) are small (80 amino acids) sphingolipid activator proteins. They are proteolytically derived from a common precursor, prosaposin. In the lysosomal sphingolipid degradation pathway, acid b glucosidase (GCase) requires saposin C for optimal *in vitro* and *in vivo* activity. The deficiency of prosaposin/saposins (PS-/-) leads to a decrease of GCase activity in selected tissues of affected humans and mice. In PS-/- mice, GCase activities were decreased by 65 to 80% in kidney and liver, and by over 50% in cultured fibroblasts and hepatocytes as determined *in vitro* with detergent assays. The GCase proteins also were decreased concordantly in these tissues and cells. GCase mRNA was at normal levels. The stability or disappearance rate of GCase in PS-/- fibroblasts was evaluated by pulse-chase metabolic labeling the cell lysates using $^{35}S$ methionine/cystine and immunoprecipitation with anti-mouse GCase antibody. Compared to the wild type cells ($t_{1/2}$> 24 h), the GCase in the PS-/- cells had faster disappearance rate ($t_{1/2}$< 1 h). Treatment of PS-/- cells with an inhibitor of lysosomal serine and cysteine proteases led to about 70% increase in GCase activity and protein. These data indicate that in the absence of saposins, GCase is unstable and proteolytically degraded in the lysosome. This leads to diminished enzyme protein and thereby reductions in cognate enzymatic activity *in vivo* in excess of the expected direct effects of saposin C alone on GCase activation. These results indicate a new property for selected saposins — an anti-proteolytic protective function for their cognate enzymes *in vivo*.

Program Nr: 55 from 2002 ASHG Annual Meeting

**Uniparental disomy of chromosome 2 resulting in lethal trifunctional protein deficiency due to homozygous alpha-subunit mutations.** *U.D. Spiekerkoetter[1], A. Eeds[2], Z. Yue[1], J. Haines[2], A.W. Strauss[1, 2], M. Summar[1, 2]*. 1) Department of Pediatrics; 2) Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN.

The mitochondrial trifunctional protein (TFP) is an enzyme complex of the fatty acid beta-oxidation cycle composed of an alpha- and a beta-subunit. The two encoding nuclear genes are located in the same region on chromosome 2 (2p23). TFP deficiency due to either alpha- or beta-subunit mutations is characterized by mutational and phenotypic heterogeneity. In two unrelated patients with lethal TFP deficiency, we delineated apparently homozygous alpha-subunit mutations that were present in heterozygous form in both mothers, but not in either biological father. We performed a microsatellite repeat analysis of both patients and their parents using seven chromosome 2-specific polymorphic DNA markers and four non-chromosome 2 markers. In both patients, two chromosome 2-specific markers demonstrated maternal isodisomy of chromosome 2. The other five chromosome 2-specific markers were non-informative in each patient. Inheritance of alleles from chromosomes 3, 4, 5, and 7 was consistent with paternity. These results explain the apparently anomalous pattern of transmission. Six of our twelve known TFP-deficient patients with alpha-subunit mutations have disease due to homozygous changes and two of them via the mechanism of uniparental disomy (UPD) (16.7%). For very rare autosomal recessive diseases, UPD may represent a common mechanism. This study emphasizes the need to confirm mutations in parents whenever possible. TFP deficiency is another disorder that has become manifest due to isodisomy of chromosome 2. This information will impact genetic counseling for these families, reducing greatly the 25% risk normally used for recessive disorders.

Program Nr: 56 from 2002 ASHG Annual Meeting

**Ranked matrix analysis of multiple gene methylation patterns in normal human tissue.** *B. Genç[1], J. Lewin[3], M. Kursar[4], C. Ivascu[2], R. Lesche[1], S. Maier[1], J. Mueller[2], K. Berlin[2], A. Olek[1]*. 1) Biomedical R&D, Epigenomics AG, Berlin, Germany; 2) Technology development, Epigenomics AG, Berlin, Germany; 3) Information sciences, Epigenomics AG, Berlin, Germany; 4) Max-Planck-Institute for Infection Biology, Berlin, Germany.

DNA methylation is the major epigenetic signal that alters transcriptional activity and is involved in the regulation of expression of a broad variety of genes. Changes in methylation patterns have been described for several genes during differentiation in a wide spectrum of cell types. To gain more insight into development, differentiation, disease susceptibility, and genome stability we developed a new ranked matrix analysis method using multiple genes to identify global patterns of methylation in the human genome. In this pilot study we map the methylation variability across the human genome using CpG-rich regions from 100 loci. We are amplifying this regions, which have been bisulphite treated, and the PCR products are sequenced directly using conventional DNA sequencing to assess the methylation status of the sites across different human tissues. The methylation pattern for each sequenced CpG positions is calculated by an analysis software considering conversion rates and using specific data normalization. The results for each CpG position can be visualized either in a position matrix (ordered by position in sequence) or as ranked matrix by using markers determined by Fisher score.

Program Nr: 57 from 2002 ASHG Annual Meeting

**Bioinformatic prediction of human imprinted genes.** *J.M. Greally, A. Friel, M. Fazzari.* Depts Med (Hematol)/Mol Genet, Albert Einstein College of Medicine, Bronx, NY.

We have previously shown that imprinted genes in the human genome reside in regions that contain an unusually low SINE content. We tested whether these data could be used predictively, using logisting regression modelling. An all subsets approach defined four sequence characteristics that were, in combination, highly predictive of imprinted loci, with 90% sensitivity and 96% specificity. A validation test on 10 control and 4 imprinted loci (*GTL2, NNAT, IGF2R, HTR2A*) correctly assigned 13/14 loci, misassigning the *NNAT* gene, which resides in a "microimprinted" domain. The test is therefore robust enough to use predictively at this stage, and will become even more powerful with the expansion of the imprinted gene sample. A whole-genome analysis is now underway. However, our early use of this predictive test has focused on two regions known to contain imprinted genes that cause human disease phenotypes. Two regions were analysed: a 2 Mb region from chromosome 14q32.2 containing imprinted genes with marked growth effects, and the 5.5 Mb region from chromosome 6q24 encoding an imprinted gene(s) causing transient neonatal diabetes mellitus. One further imprinted gene was predicted for the 14q32.2 region, while 6 new candidate imprinted genes were found in the latter domain. The whole-genome analysis will be the first comprehensive guide to the identification of these unusually regulated genes.

Program Nr: 58 from 2002 ASHG Annual Meeting

**Altered DNA methylation pattern in the transient neonatal diabetes determined by the genomic sequencing method.** *C. Diatloff-Zito[1], E. Marquis[1], A. Nicole[1], H. Labit[1], C. Junien[1], J.J. Robert[2]*. 1) Inserm U383, Groupe Hospitalier Necker Enfants-Malades, Paris, FRANCE; 2) Federation de Pediatrie, Groupe Hospitalier Necker Enfants-Malades, Paris, FRANCE.

Transient neonatal diabetes (TND) is a rare disease characterized by an absence of insulin at birth frequently associated to intrauterine growth retardation and developmental defects. Type 2 diabetes occurs in 40% of the patients. The TND candidate locus maps to 6q24.1 in a region that harbors Zac1/PLAGL1 and HYMA1 imprinted genes. The hypothesis of imprinting defects as a cause of TND has been assessed by analyzing methylation patterns of the 5-CG-3 in CpG rich sequence at the TND locus in a series of patients. The genomic-bisulfite-sequencing technology, which allows the analysis of the methylation status of individual sites, has been used. CpG islands with different epigenetic status including the differentially methylated region (DMR) have been analyzed. The DMR contains the methylation imprint marks, in normal individuals a methylated maternal allele and a paternal unmethylated allele are detected. The majority of TND patients (9/11) shows abnormal DNA methylation patterns. Two patients with a paternal chromosome 6 isodisomy showed only an ummethylated pattern. 7/9 patients having biparental inheritance showed altered methylation profiles, with for some of them a paternal uniparental methylation pattern. The DMR could be subdivided into 2 segments with evidence of distinct methylation profile alterations. Long-range epigenetic modifications could be detected in some TND patients. The data suggests that both imprinting mechanisms and methylation processes should be perturbed in TND. The loss of methylation may result from defects in imprinting establishment, DNA methylation, and may have incidence on chromatin conformation. These components are expected to influence the TND patients phenotype and outcome.

Program Nr: 59 from 2002 ASHG Annual Meeting

**An insertion/duplication mutation 11 kb upstream of Snurf-Snrpn produces a mouse model of an Angelman syndrome imprinting mutation.** *K.-S. Chen, T.-F. Tsai, A.L. Beaudet.* Molec & Human Gen, Baylor Col Medicine, Houston, TX.

The mouse 7C chromosomal region is homologous to the human chromosome 15q11-q13 Prader-Willi and Angelman syndrome critical region. In an attempt to search for an equivalent of the human AS imprinting center (AS-IC), we utilized a chromosomal engineering approach to prepare two deletions in mouse chromosome 7C upstream of *Snurf-Snrpn*: deletion A was 100 kb and extended from 11 to 111 kb and deletion B was 1.5 Mb extending from 111 kb to 1.61 Mb. Heterozygous F1 mice (chimera 129/SvEv X C57BL/6J) with paternal, but not maternal, inheritance of the 1.5 Mb deletion B demonstrated neonatal growth retardation. PCR analysis of homozygous deletion B DNA indicated that three markers (*BXR4*, *Magel2* and *Mkrm3*) flanked the deletion, while eight (*328L8T7*, *451M15SP6*, *451M15T7*, *D7MIT249*, *426B15T7*, *D7MIT276*, *476G22T7* and *Ndn*) were internal. The results indicate that the gene order in the mouse 7C region is *Mkrm3-Magel2-Ndn-SnurfSnrpn*. No obvious phenotype was observed in mice carrying the 100 kb deletion A, regardless of the parental origin. Surprisingly, maternal inheritance of the Snrpn-proximal anchor site for deletion A, (inserts puromycin cassette and 3-HPRT and duplicates 6 kb genomic DNA 11 kb upstream of exon 1 of *Snurf-Snrpn*) resulted in loss of methylation on the maternal alleles of both *Snurf-Snrpn* and *Ndn*. This is the mouse equivalent of an imprinting mutation causing AS in humans, and the result indicates the conservation of an AS-IC equivalent region in the mouse.

Program Nr: 60 from 2002 ASHG Annual Meeting

**Evidence for the role of *PWCR1/HBII-85* C/D Box small nucleolar RNAs in Prader-Willi syndrome.** *R.C. Gallagher[1], B. Pils[1,2], M. Albalwi[1], U. Francke[1]*. 1) Genetics, Stanford University, Stanford, CA; 2) Max-Planck Insitute for Molecular Genetics, Berlin-Dahlem, Germany.

Prior work in mouse and humans has provided evidence that loss of expression of one or more of the many C/D box small nucleolar RNAs (snoRNAs) encoded within the complex, paternally expressed *SNRPN* locus results in the phenotype of Prader-Willi syndrome (PWS). We suggest that the minimal critical region for PWS is roughly 121 kb of the > 460 kb *SNRPN* locus, bordered by a breakpoint cluster region within *SNRPN* identified by three individuals with PWS who have balanced reciprocal translocations, and the proximal deletion breakpoint of the unaffected mother of three children with Angelman syndrome (AS) who inherited the microdeletion from her father. These boundaries are within the chromosome 15q11-13 region at nucleotides 22057639 and 22178529, respectively (Human Genome Browser, UCSC, Dec. 2001 freeze). The subset of *SNRPN* encoded snoRNAs within this region is comprised of the *PWCR1/HBII-85* cluster of snoRNAs and the single *HBII-438A* snoRNA. These are the only known genes within this region, which suggests that loss of their expression may be responsible for much or all of the phenotype of PWS. This hypothesis is challenged by two PWS individuals who have balanced translocations with breakpoints far upstream of the proposed minimal critical region, but whose cells were reported to express transcripts within it, adjacent to these snoRNAs. By use of real-time quantitative RT-PCR, we re-assessed expression of these transcripts and of the snoRNAs themselves in fibroblasts of one of these patients. We find that the transcripts, *IPW* and *PAR-1*, that were reported to be expressed in lymphoblast-somatic cell hybrids are not expressed in fibroblasts, and suggest that the original results were misinterpreted because of the absence of a -RT control. Most important, we show that the *SNRPN* intron-encoded *PWCR1/HBII-85* snoRNAs are not expressed in fibroblasts of this PWS individual. These results are consistent with the hypothesis that loss of expression of the snoRNAs in the proposed minimal critical region confers much or all of the phenotype of PWS.

Program Nr: 61 from 2002 ASHG Annual Meeting

**A *Drosophila* Model of Fragile X premutation: CGG repeat instability and dominant-negative effects of premutation riboCGG repeat.** *P. Jin[1], F. Zhang[1], D.C. Zarnescu[2], K. Moses[2], J.C. Lucchesi[3], K. Nichol[4], C.E. Pearson[4], S.T. Warren[1]*. 1) Howard Hughes Medical Institute and Dept Human Genetics, Emory University School of Medicine, Atlanta, GA; 2) Dept Cell Biology, Emory University School of Medicine, Atlanta, GA; 3) Dept Biology, Emory University, Atlanta, GA; 4) Dept Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Fragile X syndrome is caused by an expansion of CGG repeats in the 5' UTR of the FMR1 gene. Premutation alleles (60-200 repeats) are unstable upon germline transmission with both expansions and contractions occurring. In fragile X premutation carriers, the transcription of FMR1 gene is elevated and several phenotypes have been observed specifically associated with premutation carriers, i.e., female carriers have increased incidence of premature ovarian failure while some male carriers showed a progressive neurodegenerative phenotype characterized by tremor and ataxia with the accumulation of nuclear inclusions in neurons and glial cells. To understand the molecular mechanism of repeat instability and the possible toxic effects of an elevated premutation riboCGG repeat, we have used the GAL4/UAS system to create a *Drosophila* model, in which a premutation allele (90 CGG repeats) was inserted into the 5' UTR region of GFP reporter gene under the control of UAS. In wildtype background, these transgenic flies showed moderate CGG repeat instability upon germline transmission with both expansions and contractions observed. Currently we are using this system to examine the role of several candidate genes including FEN1 in repeat instability. Ubiquitous overexpression of CGG repeats led to lethality in males and reduced viability in females. The females with high levels of the premutation riboCGG repeat displayed ovary defects and failed to produce fertilized eggs. Targeted overexpression of CGG repeat in the eye led to extensive cell death. These results suggested a dominant-negative role of overexpressed permutation riboCGG repeat and support a model that an RNA-binding protein, interacting with the riboCGG repeat of the FMR1 message, is sequestered from its normal function(s) in premutation carriers due to an elevated riboCGG repeat.

Program Nr: 62 from 2002 ASHG Annual Meeting

**SBMA motor neuronopathy in AR YAC CAG100 transgenic mice.** *B.L. Sopher[1], R.A. Martinez[1], I.E. Holm[2], A.C. Smith[1], L.-W. Jin[2], C.B. Ware[3], A.R. La Spada[1].* 1) Dept Lab Med, Univ Washington, Seattle, WA; 2) Dept Pathology, Univ Washington, Seattle, WA; 3) Dept Comp Med, Univ Washington, Seattle, WA.

Spinal and bulbar muscular atrophy (SBMA) is a lower motor neuron disease caused by a CAG repeat expansion in the androgen receptor (AR) gene. To determine the molecular basis of the neurodegeneration, we obtained a 450 kb yeast artificial chromosome (YAC) containing the ~100 kb human AR gene and targeted a CAG100 repeat into this AR YAC. Yeast carrying AR YAC CAG20 (control) or AR YAC CAG100 were fused to embryonic stem (ES) cells, selected ES cells were injected into blastocysts to yield chimeras, and independent lines of AR YAC CAG20 and AR YAC CAG100 transgenic mice were generated. RT-PCR analysis revealed levels of AR transgene expression ranging from 0.4 - 0.75 that of endogenous murine AR levels, and expression of full-length human AR protein was confirmed by Western blot. While the AR YAC CAG20 transgenic mice are phenotypically normal, we noted that AR YAC CAG100 transgenic mice show onset of a neuromuscular phenotype beginning at 8 - 12 months of age. With progression of the disease, the mice lose weight, develop a kyphotic appearance, and show decreased survival. Affected AR YAC CAG100 mice display marked hindlimb atrophy, and onset of this phenotype is accompanied by gait abnormalities, as evidenced by significantly decreased stride lengths on footprint analysis. Presymptomatic AR YAC CAG100 individuals show significant reductions in muscle strength when evaluated by the hanging wire grip strength test. Neuropathology studies of AR YAC CAG100 mice indicate that severe muscle atrophy is occurring, as numerous shrunken muscle fibers are observed intermixed with hypertrophied fibers. The overall histopathology is consistent with denervation atrophy, supporting the conclusion that independent lines of AR YAC CAG100 mice suffer from a motor neuronopathy. As the AR YAC CAG100 mice recapitulate the SBMA neuromuscular phenotype with the human AR transgene placed under proper regulatory control, we believe that this mouse model will be particularly useful in understanding the initial events along the pathway of AR polyglutamine-induced motoneuron toxicity.

Program Nr: 63 from 2002 ASHG Annual Meeting

**Myotonic Dystrophy: RNA CUG repeats and CUG-binding proteins.** *L.T. Timchenko[1], Z-J. Cai[1], P. Iakova[2], L. Qian[1], R. Patel[1], N.A. Timchenko[2].* 1) Dept Cardiovascular Sci, Baylor Col Medicine, Houston, TX; 2) Dept Pathology and Huffington Center on Aging, Baylor Col Medicine, Houston, TX.

Myotonic Dystrophy 1 (DM1) is a complex disease associated with skeletal muscle weakness, myopathy and myotonia, and with the defects in the heart, the brain, and the endocrine system. DM1 is caused by an expansion of CTG/CUG repeats located in the 3'UTR region of DMPK mRNA. Accumulation of RNA CUG repeats leads to deregulation of an RNA CUG-binding protein, CUGBP1, which in turn, causes delay of skeletal muscle differentiation (Timchenko et al, 2001, Mol Cell Biol) and an unusual resistance to insulin (Savkur et al, 2001, Nat Genet). To identify additional specific CUG-binding proteins, we performed analysis of proteins which bind to long $CUG_{123}$ repeats. In DM1 skeletal and cardiac muscles, two major $CUG_{123}$/protein complexes were observed. A faster migrating complex is formed by CUGBP1. A 60kD CUG binding protein, forming the second major complex, was purified to homogeneity, sequenced and shown to be identical to calreticulin (CRT). CRT is a multifunctional protein that possesses a ds-RNA-binding activity and plays a crucial role in heart functioning. Both CRT and CUGBP1 bind stronger to transcripts containing long CUG repeats and to stem-loop (SL) structures with GC-rich sequences in the stem. Searching for endogenous CRT targets, we found that mRNAs coding for C/EBP transcription factors contain GC-rich SL structures in the regulatory regions. CRT binds to SL structures within these mRNAs and inhibits translation of C/EBP alpha and C/EBP beta proteins in a cell-free translation system and in cultured cells. Since C/EBP proteins regulate cell growth and proliferation, we suggest that expansion of CUG repeats may also distort CRT and change proliferative status in DM1 cells via deregulation of the C/EBP transcription factors. These data support the hypothesis that the expansion of CUG repeats in DM1 affects several families of specific CUG RNA binding proteins and causes various symptoms through RNA pathway.

Program Nr: 64 from 2002 ASHG Annual Meeting

**SCA8 BAC transgenic mice have a progressive and lethal neurological phenotype demonstrating pathogenicity of the CTG expansion.** *M.L. Moseley, M. Weatherspoon, L. Rasmussen, J.W. Day, L.P.W. Ranum.* Institute of Human Genetics, Univ MN, Minneapolis, MN.

SCA8, a dominantly inherited, slowly progressive spinocerebellar ataxia, is caused by an untranslated CTG expansion located at the 3' end of a processed, non-coding transcript. How the CTG expansion causes disease and the reason for the high frequency of reduced penetrance are not understood. Parallels between the SCA8 mutation and mutations causing myotonic dystrophy suggest that pathogenic effects of the CUG expansion at the RNA level cause SCA8. To better understand disease pathogenesis we have generated a transgenic mouse model. Homologous recombination was used to replace the normal SCA8 repeat tract (11 CTGs) in a human BAC clone with a pathogenic expansion of 107 CTG repeats. The resulting BAC constructs, containing the human promoter and large flanking regions that extend 90 kb upstream and 25 kb downstream of SCA8, would be expected to contain all of the cis-acting regulatory elements needed to accurately mimic the low steady state transcript levels as well as CNS- and testis-specific gene expression found in humans. Seven lines with expansions and three with normal repeat tracts (copy # ~1-10) were obtained. SCA8 expression is detected by RT-PCR in all 7 expansion lines, and mimics the human expression pattern, with low transcript levels detectable in all CNS tissues but not in other parts of the body that were checked. Rotarod analysis was performed to assess motor performance of 26-week-old transgenic animals compared to their age-matched non-transgenic littermates. A trend towards poorer motor performance was seen in all of the expansion lines with one line (E1) showing significantly poorer performance ($p=4.7x10^{-5}$). Two of the expansion lines with the highest copy number (E1 and E7) show a progressive, severe phenotype in which animals have increased motor tone, progressive kyphosis (spinal curvature), gait instability, weight loss and death (survival time varies from 14 to >52 wks). A similar phenotype has not been observed in the transgenic animals with the normal CTG repeat tract. We are currently investigating CNS pathology.

Program Nr: 65 from 2002 ASHG Annual Meeting

**Experimental evidence for frequent polymorphic gene duplication in the human genome.** *P.R. Buckland, B. Hoogendoorn, C. Guy, S.K. Smith, S.L. Coleman, M.C. O'Donovan.* Psychological Medicine, University Wales College of Medincine, Cardiff, South Glamorgan, UK.

Duplication of DNA sequence, including entire genes, is considered to be one of the driving forces of evolution in the eukaryotic genome as the duplicated genes are free to mutate into new protein encoding genes whilst the original remains intact. A number of genes are know to have one or more paralogous copies in the genome, however, a growing number of genes have been identified that are subject to sporadic, or polymorphic duplication, frequently leading to diseases either directly due to increased copy number or indirectly by facilitating unequal crossing over leading to gene loss. As part of a large project surveying promoter polymorphisms we have identified a large number of promoter sequences which have paralogous copies in the genomes of some individuals. 700 gene promoters were selected and approximately 500 bp screened for sequence variants using DHPLC in a panel of 16 individuals from diverse ethnic backgrounds. Polymorphisms were found in over 300 of these including 100 where at least 1 individual had 2 or more polymorphisms. Each of the latter was amplified by PCR and cloned into a plasmid and used to transform SURE cells. Sequencing of the clones revealed that 65 promoters gave rise to 3 or more haplotypes originating from a single individual indicating the presence of more than two copies of the sequence in that subjects genome. This represents 9% of the genes studied. Several of these genes were found to be duplicated in all individuals studied and the sequence studied gave multiple alignments on the draft human genome (e.g. LHB and genes on 22q11.2). However the majority were only duplicated in some individuals (and single alignments were found with the draft human genome). We suggest that this represents polymorphic gene duplication which may have significant relevance to human variation and disease and may also have implications for gene linkage and association studies of complex illnesses.

Program Nr: 66 from 2002 ASHG Annual Meeting

**Performing classical statistical genetic tests in founder populations.** *C. Bourgain[1], D. Newman[1], C. Ober[1], M.S. McPeek[1,2]*. 1) Departments of Human Genetic and; 2) Statistics, The University of Chicago, Chicago, IL.

Founder populations are of growing interest for complex trait mapping. Expected reductions in the number of segregating alleles, more extended linkage disequilibrium and homogenous environments should help to identify complex genetic risk factors. However, studies in such populations present several statistical issues. In particular, the relatedness among individuals may be considerable, leading to non-independent observations. Consequently, the type I error of classical statistical tests such as case-control association tests or the Hardy-Weinberg (HW) test may dramatically increase, but the pedigree structure may be too complex to use maximum likelihood based tests, because all individuals may be related through multiple lines of descent. We propose a new quasi-likelihood approach based on the condensed identity coefficients to assess the covariance of observations between any pair of individuals. This framework is used to construct a quasi-likelihood score test for HW, called QL-HW. This test, performed conditional on the pedigree structure, can be used to detect deviations from HW that may result from genotyping errors or selection pressure. In the Hutterites of South Dakota, a population derived from 64 ancestors, extensive pedigree information is available and the nine identity coefficients for each pair of individuals have been computed (Abney et al. Am J Hum Genet. 2000). Statistical properties of the QL-HW test were studied in this population. 143 SNPs in 82 genes were tested in a sample of > 600 Hutterites. Significant departure from HW at the 5% level was observed for 14 SNPs, including 4 SNPs in the HLA region. A single SNP, located in a positional candidate gene for asthma on chromosome 19, still showed significant evidence of departure from HW expecting after correcting for multiple (82) tests. We are currently evaluating whether these results reflect genotyping errors or selection effects. This approach can be extended to construct a case control association test, which should be more powerful than the TDT in populations where stratification is absent, such as in the Hutterites. Supported by HL49596.

Program Nr: 67 from 2002 ASHG Annual Meeting

**Inference of population structure in recently admixed populations.** *J.K Pritchard[1], D. Falush[2], M. Stephens[3].* 1) Dept Human Genetics, Univ Chicago, Chicago IL; 2) Department of Molecular Genetics, Max-Planck Institut fuer Infektionsbiologie, Berlin, Germany; 3) Department of Statistics, University of Washington, Seattle, WA.

There are numerous settings in which it is important to know about population structure, and the population membership of individuals in a sample. This is relevant in association mapping and admixture mapping, as well as in evolutionary studies, and studies of human history. In earlier work (Pritchard, Stephens and Donnelly, Genetics 2000), we introduced a model-based clustering scheme that uses multilocus genotype data from a sample of individuals to detect the presence of population structure, and to estimate the ancestry of the sampled individuals. The earlier method assumed that loci were independent; however this assumption is likely to be violated when studying admixed populations, where there can be correlations even between weakly linked loci. In view of the considerable interest in admixed populations, we describe a new model that accounts for the correlations between linked loci that arise in admixed populations (i.e. admixture linkage disequilibium). This modification has several advantages, allowing (1) detection of admixture events further back into the past; (2) inference of the population of origin of chromosomal regions; (3) more accurate estimates of statistical uncertainty when linked loci are used. These new methods are implemented in a program, Structure, Version 2, that is available at http://pritch.bsd.uchicago.edu.

Program Nr: 68 from 2002 ASHG Annual Meeting

**High resolution genetic maps and complete genealogy of an isolated Sardinian sub-population as prerequisite for the study of complex traits.** *M. Pirastu[1], P.M. Melis[1], A. Angius[1], P. Forabosco[1], M. Falchi[1], E. Petretto[1], C. Cappio Borlino[2], P. Unali[2], G. Mancosu[2], P. Ledda[2], C. Hayward[3], A.F. Wright[3].* 1) Istituto di Genetica delle Popolazioni, CNR, Alghero, Sassari, Italy; 2) SharDNA, Cagliari, Italy; 3) MRC Human Genetics Units, Edinburgh, UK.

Founder isolated populations, particularly ones with a large, known genealogy and reduced environmental noise, present potential advantages for complex-trait mapping studies using LD-mapping and linkage approaches. We selected a particularly isolated area of Sardinia and started our project in one village of this region, Talana. Archival records allowed the tracing of all relationships among the 1200 extant individuals up to 1600. We have complete genealogical information of Talana in the form of a large pedigree of 5219 individuals. All data have been stored in a newly developed database, which allows both visual inspection and automatic extraction of the data for analysis. Additionally, we have extensive phenotypic characterization and a 2 cM microsatellite map for 895 individuals, genotyped by the NHLBI Mammalian Genotyping Service and the MRC Genetic Unit of Edinburgh. Markers heterozygosity is still very high (0.72, SD=0.09) even in such a highly inbred population. We focus our study on the subset of genotyped subjects, whose complete relationships among each other form a 16-generation pedigree of 2104 individuals with multiple inbreeding loops. The mean number of meiotic steps connecting these subjects is 8.2 (SD=2.6) through six main minimum ancestors. The estimated mean kinship coefficient is 0.011 (SD=0.020) and the mean inbreeding is 0.009 (SD=0.013). There is growing evidence that recombination rates along chromosomes differ among individuals and populations. Using the large number of informative meioses derived from genealogical and genotypic data, we have estimated the population-specific genetic maps and compared it with reference physical maps in order to evaluate the distribution of hot and cold spot recombination sites along the whole genome. Given the increased level of LD observed in the population, blocks of LD were also investigated and compared with the distribution of recombination events.

Program Nr: 69 from 2002 ASHG Annual Meeting

**Genetic Variation in the Conservative Mennonites of Eastern Pennsylvania.** *A. Chakravarti[1], M.M. Carrasquillo[1], C. Kashuk[1], E.G. Puffenberger[2].* 1) Inst Genetic Medicine, Johns Hopkins Hosp, Baltimore, MD; 2) Clinic for Special Children, Strasburg, PA.

Isolated populations have genetic variation patterns which have been significantly affected by genetic drift. There is widespread expectation that heterozygosity is reduced and linkage disequilibrium is increased. Consequently, isolated communities of recent origins are excellent populations for mapping genes underlying complex phenotypes using allelic association. We have studied in excess of 590 autosomal, X-linked, and Y-linked microsatellite markers, 1450 single nucleotide polymorphisms (SNPs), as well as nucleotide sequencing of the mtDNA HVR I/II, in ~ 40 chromosomes in the Old Order Mennonites of Lancaster and Berks Counties, PA. These data were compared to ~40 chromosomes from the Utah set of CEPH families. We show that gene diversity (h), is significantly reduced in the Mennonites on autosomes, but not on the X chromosome. SNPs show generally the same pattern. Analyses of allele frequency differentiation using FST suggest an effective population number of ~ 100. The genetic data are consistent with a larger number of founding females than males, consistent with Mennonite genealogies. We constructed a common microsatellite and SNP linkage map based on the reference genome sequence to evaluate linkage disequilibrium of adjacent marker loci. We demonstrate significantly greater association within Mennonites (~15%) as compared to CEPH (threshhold of p<5%) with association being inversely related to inter-marker map distance. We use these data to estimate the probability of mapping by allelic association in the Conservative Mennonites.

Program Nr: 70 from 2002 ASHG Annual Meeting

**Accelerated Genetic Drift by a Heritable Component of Family Size in a Founder Population.** *D.K. Nolan[1], A. Pluzhnikov[2], X. Dong[3], Z. Tan[3], M.S. McPeek[3,2,1], C. Ober[2,1]*. 1) Committee on Genetics, U. of Chicago, Chicago, IL; 2) Dept. of Human Genetics, U. of Chicago, Chicago, IL; 3) Dept. of Statistics, U. of Chicago, Chicago, IL.

Our previous study of mitochondrial and Y chromosome polymorphisms in the Hutterites, a founder population of European origin, revealed significantly greater genetic drift than expected given their pattern of exponential growth (Nolan et al. AJHG 69: A257, 2001). This drift was evidenced by a loss of paternal and maternal lineages since the founding of the population in the early 1700's: only 3 out of 50 female and 3 out of 38 male founders in this genealogy accounted for >60% of all paternal and maternal lineages. This could result from a positive parent-offspring correlation in effective family size (EFS), defined as the number of reproducing offspring per reproducing woman. A positive EFS correlation can accelerate drift and increase the frequency of high fertility lineages, resulting in skewed founder contributions and reduced effective population size. A positive EFS correlation (r=0.14) was observed in a French-Canadian founder population, where it was attributed to cultural factors (Austerlitz & Heyer PNAS 95: 15140, 1998). To determine whether EFS correlations contribute to drift in the Hutterites, we identified 3-generation families using the entire 12,903 person 13-generation S-leut pedigree. We restricted the birth date of the grandmother to 1860 or earlier to reduce bias due to incomplete EFS. The EFS correlation was estimated in 231 families, yielding r=.43, considerably higher than that in the French-Canadians. A correlation similar to the French-Canadian value was obtained (r=.18) when families with incomplete EFS were included. Methods that take into account kinship are being developed for assessing the significance of this correlation. Nonetheless, because of their communal and relatively homogeneous lifestyle, it is less likely that cultural factors play a significant role, suggesting a heritable component of family size in the Hutterites. The high correlation in EFS values could explain the observed loss of genetic diversity in this population. Supported by HD21244.

Program Nr: 71 from 2002 ASHG Annual Meeting

**_P_-gene (OCA2) haplotype variation and association with skin color.** *R.A. Kittles[1], A. Massac[1], G. Vosganian[1], W. Chen[1], M. Doura[1], A. Zhao[1], R. Halder[2], E. Parra[3], M. Shriver[3].* 1) National Human Genome Center at Howard University, Washington, DC; 2) Dept. of Dermatology, Howard Univ. Hospital; 3) Dept. of Anthropology, Penn State University, State College, PA.

Very little is known regarding the genetics of skin color despite its conspicuous nature and high heritability. An interesting candidate gene is the *P*-gene which controls pH within the melanosome. Here we present data on *P*-gene variation in unrelated individuals from five populations. SNPs within exons 9 (R222W C/T), 10 (A272A G/A), 20 (A603A A/C), and 23 (A693A C/T) were genotyped using Pyrosequencing in 256 African Americans (AA), 186 European Americans (EA), 33 Hispanics (HP), 100 Nigerians (NI), and 60 Asians (AS). Skin color was assessed for AA, EA, and HP volunteers by reflectance spectroscopy using the DermaSpectrophotometer (cyberDerm, PA). LD was assessed between pairs of SNPs separated from 3.4 kb to 113 kb and haplotypes were estimated by the EM algorithm. MANOVAs and ANCOVAs were performed to test genotype, haplotype, age, and sex effects on skin color and to control for admixture among African Americans. Significant *P*-gene differentiation was observed among populations ($F_{ST}$=0.19). Two common haplogroups were observed with large frequency differences (d =0.60) between Africans and non-Africans. Almost 50% of all haplotypes (6 of 13) were only observed in populations of African descent. LD extended furthest (~114kb) among African Americans due to admixture disequilibrium. ANOVAs revealed significant associations between skin color (M index) and SNP A272A in African Americans (P=0.0003) and SNP A693A in Europeans (P=0.009) and Hispanics (P=0.04). Both of these SNPs were associated with increased melanin levels. No significant effects of age and gender on skin color was observed (P>0.09). When individual admixture was included as a covariate in the analysis for African Americans the A355A SNP was still strongly associated with skin color (P=0.008). From the haplotype data we estimate that *P*-gene variation may account for about 15% of the genetic variance of skin color. These results have important significance for research on skin cancer susceptibility.

Program Nr: 72 from 2002 ASHG Annual Meeting

**Markers Distinguishing Ancestry Demonstrate Strong Extended Linkage Disequilibrium in African Americans.**

*M.F. Seldin[1], B.S. Chima[1], D.J. Operario[1], J.L. Weber[2], L.A. Criswell[3], R.S. Cooper[4], H.E. Collins-Schramm[1]*. 1) Rowe Program in Human Genetics, UC Davis, Davis, CA 95616; 2) Marshfield Medical Research Foundation, Marshfield, WI 54449; 3) Department of Medicine, UC San Francisco, San Francisco, CA 94143; 4) Preventive Medicine and Epidemiology, Loyola Univ, Maywood, IL 60153.

Conflicting reports regarding the extent of linkage disequilibirum (LD) in the African American (AA) population have created uncertainty about the usefulness of admixture mapping in this population. By examining regions of human chromosomes 5 and 22, we demonstrate that significant and impressive LD is observable for extensive distances in AA for diallelic markers with large allele frequency differences (measured by the f value) between European Americans and Africans. In contrast, only rare, marginally significant LD is present between unlinked markers in this population. Significant LD extends up to 12 cM (e.g. MID1191 at 126.2 cM and MID719 at 138.4 cM on Chr 22, $p=10^{-4}$, D'=0.54). In representative founder populations, strong LD was only observed for markers separated by less than 10kb and suggestive LD was only observed at greater distances in a single comparison in the African population (MID1102 at 135.1 and MID768 at 135.5, p= 0.003, D'=0.26). For unlinked markers, only one of the 42 pair-wise analyses in African Americans was associated with a p value < 0.01 (MID619 and MID883, p=0.003, D'= 0.2). Examination of the strength of LD demonstrated that the ability to detect LD for extended chromosomal segments decays as a function of both the f values and distance between markers. Unlinked marker comparisons are clearly distinguishable from linked marker comparisons by LD strength. It is likely that the conflicting results of previous reports are due to differences in the f values of markers chosen for analysis. These results indicate that obtainable markers with large f values can detect extended regions of LD and should therefore be useful in admixture mapping in the AA population.

Program Nr: 73 from 2002 ASHG Annual Meeting

**Paternal lineages of African Americans in South Carolina.** *M.B. Doura, R.A. Kittles.* National Human Genome Center at Howard University, Washington DC.

The early African experience in the Americas is marked by the transatlantic slave trade from ~1619 to 1850 and the rise of the plantation system. Plantation systems and the origins of enslaved Africans differed geographically. In colonial South Carolina, plantations were largely viewed as rice based this period. The South Carolina plantation complex has been characterized by a preference for enslaved Africans from the Upper Guinea 'Rice' Coast of West Africa (Senegal to Sierra Leone). However this hypothesis has received limited support from cultural and linguistic studies. Here we present genetic evidence for Upper Guinea Coast ancestry by examining Y chromosomes in African American males from Columbia, S.C. Y chromosome haplotypes were constructed from eight Y specific STRs, an ALU insertion, and a SNP typed in 244 unrelated African Americans, 344 West Africans (11 populations from Senegal, Gambia, Guinea, Sierra Leone, Liberia, Nigeria and Cameroon), and 88 European Americans.

A total of 414 distinct haplotypes were observed with 319 (77%) being unique to one male. Phylogenetic and principal component analyses revealed significant sharing and clustering of haplotypes among African Americans and males from Sierra Leone, and Senegal. Other West African populations possessed quite divergent Y haplotypes. Admixture analysis revealed that 55% of the patrilineages originated from Sierra Leone and Senegal populations. In addition, 23-29% of African American males possess paternal lineages of European ancestry. This is in contrast to the low frequency of European mtDNA lineages among African Americans revealed by previous studies. Our results of significant Upper Guinea Coast ancestry of African Americans in Columbia, SC, and diverse paternal lineages in west Africa may have significant implications for gene mapping efforts in African American populations.

Program Nr: 74 from 2002 ASHG Annual Meeting

**Demographic history of Khoisan speakers of Tanzania inferred from mtDNA control region sequences.** *M.K. Gonder[1], H. Mortensen[1], J.B. Hirbo[1], J. Mountain[2], S.A. Tishkoff[1]*. 1) Dept. of Biology, University of Maryland, College Park, Maryland 20742; 2) Dept. of Anthropological Sciences, Stanford University, Stanford, California 94305.

The Hadza and Sandawe are unique among the ethnic groups of Tanzania because they speak languages containing click consonants, and they have traditionally subsisted through hunting and gathering. Although linguists have classified both languages as Khoisan, this classification has proven to be controversial. Furthermore, little is known about their interactions with neighboring populations practicing different subsistence methods. Thus, the origin of these populations and their relationships with their neighbors and with southern African Khoisan-speaking populations remains a genetic and linguistic puzzle.

Here, we present a large sample of complete mtDNA control region sequences obtained from Hadza and Sandawe (n > 200). These mtDNA sequences were compared to mtDNA control region sequences obtained from public genetic databases and to control region sequences obtained from neighboring Tanzanian populations (n>250). These sequences were analyzed using several phylogenetic and population genetic methods. These analyses were used to 1) characterize genetic variation among the Hadza and Sandawe compared to neighboring populations 2) to reconstruct past relationships of these East African populations to one another and to other African populations, particularly southern African Khoisan-speakers and 3) to reconstruct modern human origins. Preliminary analyses of these data indicate very high levels of genetic diversity and deep mtDNA lineages for the Hadza and Sandawe and moderate levels of gene flow among populations. Genetic and linguistic affinity did not correspond in all instances. Funded by Burroughs Welcome Fund and David and Lucille Packard Career Awards, Wenner Gren and Leakey Foundation grants, and NSF grant BCS-9905396 to SAT.

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Program Nr: 75 from 2002 ASHG Annual Meeting

**Ancient human migrations indicated in global SNP/STR haplotype distributions.** *J.L. Mountain[1,2], I. Udina[3], C. Gignoux[1], M. Jobin[1], A.A. Lin[2], P.A. Underhill[2], L.L. Cavalli-Sforza[2], A. Knight[1], L.A. Zhivotovsky[3].* 1) Dept Anthropological Sciences, Stanford Univ, Stanford, CA; 2) Dept of Genetics Stanford Univ, Stanford, CA; 3) N I Vavilov Inst of General Genetics, Russian Academy of Sci, Moscow, Russia.

Current human population genetic structure reflects ancient and recent migrations as well as the ever-changing levels of isolation between populations. Available genetic and non-genetic data indicate that the current structure has arisen within the last one hundred thousand years. Questions remain regarding the timing of migrations and the impact of barriers to gene flow. Haplotype frequencies are particularly informative regarding human migration and isolation. Here we focus on short autosomal haplotypes that provide data complementary to and independent of Y chromosome and mtDNA haplotypes. We call the combination of a single nucleotide polymrophism (SNP) tightly linked to a short tandem repeat (STR) polymorphism a “SNPSTR”, and have developed a simple, rapid method for empirically determining gametic phase for double and triple heterozygotes. We have typed two such SNPSTR systems in a global set of populations including Siberian, Northeast, Southeast and Southwest Asian, South American, as well as several African, European, Middle Eastern and Oceanic groups. These data indicate a West-East gradient, with reduced STR diversity on each SNP background and greater linkage disequilibrium in populations sampled in the Americas and Oceania. Remarkably, population relationships inferred from this set of two genetic systems are consistent with those indicated by much larger datasets consisting of either SNP or STR polymorphisms alone. The small number of population or region specific haplotypes are particularly informative. Overall the data are consistent with a West to East spread of anatomically modern humans from Africa, with subsequent isolation of groups at eastern edges such as in the Americas. These data provide evidence of the power of autosomal SNPSTR systems to reveal the history behind current population genetic structure. Supported by NIH grant GM28428.

Program Nr: 76 from 2002 ASHG Annual Meeting

**Lathosterol 5-desaturase Deficiency: An Inborn Error of Human and Murine Cholesterol Biosynthesis.** *F.D. Porter[1], C.A. Wassif[1], D. Cozma[1], L. Kratz[2], M. Tsokos[3], A.G.W. Hunter[4], R.I. Kelley[2], P.A. Krakowiak[1].* 1) HDB, NICHD, NIH, Bethesda, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) NCI, NIH, Bethesda, MD; 4) Children's Hospital of Eastern Ontario, Ottowa, ON.

Inborn errors of cholesterol synthesis underlie a group of human malformation syndromes including Smith-Lemli-Opitz syndrome (SLOS), desmosterolosis, CHILD syndrome, and CDPX2. We now report the identification of a human lathosterolosis patient and the characterization of a mouse model. Lathosterolosis is due to a deficiency of lathosterol 5-desaturase (SC5D). SC5D catalyzes the conversion of lathosterol to 7-dehydrocholesterol (7-DHC). This enzymatic step immediately precedes the defect in SLOS. A human patient, who had previously been described as an atypical case of SLOS with mucolipidosis, was identified. Biochemical analysis showed that fibroblasts from this patient accumulated lathosterol rather than 7-DHC. Cholesterol synthesis was 32% of total sterol synthesis. Molecular analysis of this patient, with heterozygostity confirmed in his parents, demonstrated a homozygous mutation of *SC5D* (137A>C, Y46S) at a highly conserved amino acid. This child had craniofacial abnormalities including micrognathia as well as limb patterning defects which included both 2-3 toe syndactyly, postaxial polydactyly of the feet, and ambiguous genitalia. *Sc5d* was disrupted in mouse embryonic stem cells. *Sc5d+/-* mice are phenotypically normal. Biochemically, *Sc5d-/-* embryos had elevated tissue lathosterol levels and decreased cholesterol levels. *Sc5d-/-* pups were stillborn, had craniofacial defects including a Robin sequence with cleft palate and micrognathia, and had both proximal-distal as well as preaxial-postaxial limb patterning defects. Many of the malformations found in these mice are consistent with impaired hedgehog signaling, and appear to be a result of decreased cholesterol rather than increased lathosterol. An unique aspect of the lathosterolosis phenotype is its presentation as a malformation syndrome with lysosomal storage. Analysis of both $SC5D^{Y46S/Y46S}$ and *Sc5d-/-* fibroblasts showed that lysosomal storage is a characteristic of both the human and murine lathosterolosis.

Program Nr: 77 from 2002 ASHG Annual Meeting

**The Phenotypic Expression of Bardet-Biedl Syndrome (BBS) by Genotype: Prevalence of Cardinal Manifestations.** *E. Dicks[1], S. Moore[1], J. Green[1], B. Fernandez[2], Y. Fan[3], A. Bhogal[3], W. Davidson[3], D. Macgregor[2], L. Penney[2], P. Parfrey[1]*. 1) Memorial University of Newfoundland, St John's, NF, Canada; 2) Newfoundland and Labrador Medical Genetics Program, St John's, NF, Canada; 3) Simon Fraser University, Vancouver, BC, Canada.

The cardinal manifestations of BBS (OMIM:209900) are retinal dystrophy, obesity, renal abnormalities, dystrophic extremities and hypogenitalism. To assess the prevalence of these in a cohort of 46 BBS patients (ages 1 to 67 years) from 26 families in Newfoundland, medical histories were reviewed and 25/46 patients were examined in 2001. Six patients from 5 families were linked to the BBS1 locus on 11q13, one to 16q21 (BBS2), 5 patients from 1 pedigree linked to 3p12 (BBS3), and 5 patients from another family linked to 2q31 (BBS5). In a further 12 patients from 6 families, a mutation was identified in the *MKKS* gene associated with BBS6. The genotype was unknown in the other 17/46 (37%) patients from 12 families (2 patients from 1 family were excluded from all loci). All except 3 patients (ages 1-7 years) were registered blind. Obesity (BMI > 27 kg/m2) occurred in all except one at some time. Renal imaging abnormalities, including fetal lobulation, dilated calyces and cysts were seen in 27/28 (96%). Polydactyly was present in 29/46 (63%), 30/35 (86%) had syndactyly and 34/35 (97%) had brachydactyly. All 5 BBS5 patients had no polydactyly, within the other genotypes this feature was very variable. Genital abnormalities included small penile length in 20/21 (95%) males. 2/20 (10%) females had vaginal atresia and 12/19 (63%) had an irregular menstrual cycle. 26/46 (56%) had IQ testing, the IQ was below 80 in 15/26 (58%), and below 50 in 2/26 (8%). There was no significant difference in IQ with genotype. The lack of a phenotype-genotype correlation for these manifestations supports the hypothesis that the BBS genes are integral to the same developmental pathway.

Program Nr: 78 from 2002 ASHG Annual Meeting

**Genotype-phenotype analysis in patients with X-linked lissencephaly with abnormal genitalia (XLAG), X-linked infantile spasms and X-linked mental retardation.** *M. Kato[1], Y. Peng[1], E. Kretzschmar[1], P. Chen[1], G. Raca[1], K. Kitamura[2], N. Sugiyama[3], T. Fukuda[3], K. Morohashi[3], S. Das[1], W.B. Dobyns[1]*. 1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Mitsubishi Kagaku Institute of Life Sciences, Tokyo, Japan; 3) Department of Developmental Biology, National Institute for Basic Biology, Okazaki, Japan.

We recently identified *ARX* as the gene responsible for X-linked lissencephaly with abnormal genitalia (XLAG) in males and agenesis of the corpus callosum (ACC) in females (Kitamura et al. submitted). Mutations of *ARX* also cause X-linked infantile spasms (ISSX) and mental retardation (MRX) (Bienvenu et al. 2002; Strfmme et al. 2002). We performed mutation analysis in 15 probands with XLAG and their relatives and performed genotype-phenotype analysis in all patients including those reported previously. In all, 21 mutations were found in 12 sporadic patients and 22 probands of multiplex families. This included 9 null mutations (large deletions, frame shifts, nonsense mutations and inactivated splicing) predicting protein truncation in exons 1-4 that caused XLAG in 10 genotypic males and ACC in one female. Heterozygous mutations were found in 7 of 10 mothers tested. At least three carrier mothers had normal brain MRI, including one with preliminary evidence of mosaicism. Deletion of exon 5 caused severe ISSX without lissencephaly or ACC in 2 males (Strfmme et al. 2002). Nonconservative missense mutations within the homeodomain caused less severe XLAG with pachygyria rather than agyria, partial ACC and normal external genitalia in 3 males, while a conservative substitution in the homeodomain caused X-linked myoclonic epilepsy with mental retardation and spasticity. Missense mutations outside of the homeodomain caused MRX in 3 families (Bienvenu et al. 2002). In-frame expansion of a GCG repeat coding for polyalanine in exon 2 caused ISSX when expanded by 7 alanine residues and MRX when expanded by 2 alanine residues. Duplication of another nearby polyalanine tract caused even less severe ISSX, Partington syndrome (mental retardation and dystonia) or MRX in 11 families. In conclusion, the type and location of *ARX* mutations predict the phenotype.

Program Nr: 79 from 2002 ASHG Annual Meeting

**Clinical and molecular genetics and epigenetics of Beckwith-Wiedemann syndrome (BWS).** *W.N. Cooper[1], K.J. Wagner[1], R. Curley[1], A. Luharia[2], J. Engel[1], P.N. Schofield[3], F. Macdonald[2], W. Reik[4], E.R. Maher[1].* 1) Medical and Molecular Genetics, The Medical School, University of Birmingham, Birmingham, B15 2TT, UK; 2) West Midlands Regional Genetics Service, Birmingham Womens Hospital, Edgbaston, Birmingham, B15 2TG, UK; 3) Department of Anatomy, University of Cambridge, UK; 4) Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge, CB2 4AT, UK.

BWS is a model imprinting disorder resulting from mutations or epigenetic events involving imprinted genes at chromosome 11p15.5. Thus germline mutations in CDKN1C, uniparental disomy (UPD) and loss of imprinting (LOI) of IGF2 and other imprinted genes have been implicated. Many (~40%) familial BWS cases have germline CDKN1C mutations. However, most BWS cases are sporadic and UPD or putative imprinting errors predominate in this group. Sporadic cases with putative imprinting defects may be subdivided into (a) those with H19 hypermethylation and silencing and LOI of IGF2 in whom it is postulated that there is a defect in a distal 11p15.5 imprinting control element (designated BWSIC1) and (b) those with loss of methylation at KvDMR1, LOI of LIT1 and variable LOI of IGF in whom it is postulated that there is a defect at a more proximal imprinting control element (BWSIC2). We investigated genotype/epigenotype-phenotype correlations in 163 cases referred for molecular investigation of BWS. Hemihypertrophy was strongly associated with UPD ($p<0.0001$), whereas exomphalos was associated with BWSIC2 defect or CDKN1C mutation and not UPD or BWSIC1 ($p<0.0001$). Combining our data with other large studies revealed that the risk of embryonal tumours was highest (>20%) for UPD and BWSIC1 cases. However while <5% of BWSIC2 and CDKN1C cases developed tumours, tumours occurred in each molecular subgroup. Investigations to determine if there is a correlation between extent of segmental disomy and tumour risk in UPD cases are in progress.

Program Nr: 80 from 2002 ASHG Annual Meeting

**Intron 4 mutation in APC gene results splice defect and attenuated FAP phenotype.** *D.W. Neklason[1,2], C.H. Solomon[1], A.L. Dalton[1], R.W. Burt[1,3].* 1) Huntsman Cancer Inst; 2) Dept. Oncological Sciences; 3) Dept. Medicine, Univ Utah, Salt Lake City, UT.

Familial adenomatous polyposis (FAP) is characterized by the early age onset of hundreds of colonic adenomatous polyps and a virtual certainty of colon cancer unless the colon is removed. Polyps appear at an average age of 16 years and cancer at an average age of 39 years. Mutations in the APC gene are found in about 90% of these cases. An attenuated form of FAP (AFAP), resulting from mutations in the far 5' (5' of codon 158) or far 3' (3' to codon 1596) ends of the gene and in select areas of exon 9, is characterized by fewer adenomas, later onset of adenomas and cancer, a decreased lifetime cancer risk, phenotypic variability within families, and a predominance of proximal colonic adenomas. A moderate form of FAP has also been suggested to result from mutations in codons 179 to 625. We report a patient referred to our Familial Colon Cancer Clinic with a history of greater than 50 adenomatous polyps at age 37, predominately in the cecum of the colon. An insertion of a T, between the second and third base pair of intron 4 of the APC gene was identified. The mutation is de novo, not present in either parent or 2 siblings. Lymphoblasts from this patient were fused to mouse E2 cell lines to generate hybrid cells harboring a single copy of human chromosome 5 (conversion technology). cDNA was generated from total RNA extracted from patient lymphoblasts and hybrid cell lines containing each APC allele. PCR amplification of the cDNA produced two products of different length corresponding to each allele. Sequencing of the PCR products revealed aberrant splicing in the mutant mRNA whereby exon 4 is deleted and exon 3 is joined to exon 5. The translational reading frame is shifted after codon 140 and a translational stop is generated predicting a truncated protein of 147 amino acids. This moderate case of AFAP provides an additional piece to the puzzle, do mutations in the APC gene give rise to two genetically and clinically distinct syndromes, FAP and AFAP, or is it a continuum?

Program Nr: 81 from 2002 ASHG Annual Meeting

**The 18q- Phenotype: Based on a Comprehensive Clinical Assessment of 90 Individuals.** *J.D. Cody[1], R.L. Schaub[1], M. Semrud-Clikeman[2], L.J. Hardies[3], J. Lancaster[3], P.T. Fox[3], W.G. Beck[4], R.L. Stratton[1], S. Shapira[1], J. Baillargeon[1], R.J. Leach[1], C.I. Kaye[1], D.E. Hale[1]*. 1) Pediatrics, UT Health Science Center, San Antonio, TX; 2) Educational Psychology, Univ. of Texas, Austin; 3) Research Imaging Center, UT Health Science Center, San Antonio, TX; 4) Dept. of Otolaryngology, UT Health Science Center, San Antonio, TX.

Deletions of chromosome 18q are among the most common of the survivable autosomal deletions, with an estimated incidence of 1/40,000 live births. Ninety individuals have participated in a comprehensive clinical assessment at The Chromosome 18 Clinical Research Center. These individuals range in age from 3 months to 32 years. We have performed the following studies: auxology, endocrinologic testing, magnetic resonance brain imaging, behavioral audiology, neurologic assessment, neuropsychological appraisal and psychiatric evaluations. We have found a very wide range of abilities and disabilities in these individuals. For example, we have assessed individuals with performance IQ scores ranging from immeasurably low to above average (120). The most common features are dysmyelination of the brain (97%) , expressive speech delay (91%), hypotonia (79%), foot deformities(74%), hearing loss (70%) and short stature (68%). Most of these features can be associated with specific regions of hemizygosity. In addition, we have observed that many of the adults have had much better outcomes than might be predicted from their early childhood progress.

Program Nr: 82 from 2002 ASHG Annual Meeting

**Utility of the intron 8 polythymidine tract in predicting phenotypes for R117H and R117C *CFTR* alleles.** *E.M. Rohlfs, E.A. Sugarman, R.A. Heim, B.A. Allitto.* Genzyme Genetics, Framingham, MA.

The phenotypic expression of the R117H *CFTR* allele is highly variable. The variable phenotypes result in part from the length of the intron 8 polythymidine tract (polyT) in *cis* with the R117H allele. We report polyT status in 46 individuals with R117H and a severe cystic fibrosis (CF) allele who were affected with CF, congenital absence of the vas deferens (CAVD) or were apparently healthy adults detected at the time of testing. Our data did not distinguish between pancreatic sufficient or insufficient CF, nor did we consider individuals with R117H and a mild/variable mutation. Of the 46 individuals, 20 were CF patients and 11 were apparently healthy adults (18-63 yrs). Individuals with 5T were 90 times more likely to be affected with CF than those with 7T (OR=90.0; 95% CI: 7.2 to 1121.3). The male:female ratio was 1.0:1.5 in CF patients and 1.0:2.7 in the healthy adults, probably reflecting the occurrence of CBAVD in males with a 7T. Comparison of polyT status in males (R117H/severe allele) with CF (n= 8) or CBAVD (n=15), indicated that those with 5T were 42 times more likely to be affected with CF than those with 7T (OR=42.0; 95% CI: 3.2 to 556.8). Another mutation at the same site, R117C, is also known to cause CF but the effect of the polyT tract on disease expression is not known due to limited data. This mutation was detected in individuals with CF, with a suspected diagnosis of CF and with infertility but was not detected in apparently healthy carriers of two CF mutations. In our experience, the frequency of R117C is 0.2% in Caucasian CF patients and 0.4% Caucasian carriers. Our analysis indicates that R117C is found predominantly on a 7T background (n=13). One individual with one copy of R117C carried 5T and 9T alleles. Parental samples were not available to set phase. At this time determination of polyT status in R117C carriers appears to be of limited utility. These data represent the largest number of individuals with R117H and a severe CF mutation in whom polyT status has been determined and support previous reports of the effect of polyT status on CF phenotypes.

Program Nr: 83 from 2002 ASHG Annual Meeting

**Hemochromatosis and Iron Overload Screening (HEIRS) Study. Prevalence of elevated iron test levels by *HFE* genotype.** *E.L. Harris[1], B.G. Mellen[2], P.C. Adams[3], R. Acton[4], J.C. Barton[4], G.D. McLaren[5], F. Dawkins[6], J.H. Eckfeldt[7], V.R. Gordeuk[6], L. Lovato[2], C.E. McLaren[5], N. Press[8], P. Sholinsky[9], M. Speechley[3], E. Thomson[9].* 1) Kaiser Permanente Center for Health Research, Portland, OR; 2) Wake Forest University, Winston-Salem, NC; 3) London Health Sciences Centre, London, Ontario; 4) University of Alabama, Birmingham, AL; 5) University of California, Irvine, CA; 6) Howard University, Washington, DC; 7) University of Minnesota, Minneapolis, MN; 8) Oregon Health & Science University, Portland, OR; 9) NHLBI (PS) & NHGRI (ET), Bethesda, MD.

The HEIRS Study is designed to evaluate the prevalence, genetic and environmental determinants, and potential clinical, personal and societal correlates of iron overload (IO) and hemochromatosis (HH) in a multi-ethnic primary care-based sample of 100,000 adults age 25 or older. Initial testing includes transferrin saturation (TS), serum ferritin (SF), and C282Y and H63D mutations of the *HFE* gene. Participants complete questions on demographics, general health, and attitudes towards genetic testing. They are invited for a clinical exam (CE) if they have C282Y homozygosity or elevated TS and SF (TS>50% and SF>300 ug/L for men, TS>45% and SF>200 ug/L for women). Results: We present results for the first 20,130 participants; 50% are Caucasian, 24% African-American, 12% Asian, 11% Hispanic, and 2% other, mixed, or unidentified race. The prevalence of C282Y homozygosity is 1/322 overall and 1/169 in Caucasians. To estimate the prevalence of elevated iron levels by *HFE* genotype, we excluded 335 participants who reported a previous IO or HH diagnosis. Initially elevated TS and SF levels were present in 55% (95% CI: 38-71) of C282Y/C282Y, 9.8% (6.2-15) of C282Y/H63D, 4.9% (2.7-8.3) of H63D/H63D, 2.4% (1.6-3.5) of C282Y/-, 1.8% (1.4-2.3) of H63D/-, and 1.5% (1.3-1.7) of -/-. The effects of race/ethnicity, age, and gender on these estimates are being assessed. From the CE we will be able to make provisional diagnoses of primary IO to estimate IO prevalence by genotype, and assess IO-related morbidity.

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Program Nr: 84 from 2002 ASHG Annual Meeting

**Apolipoprotein E controls the risk and age at onset of Parkinson disease.** *J.M. Vance[1,2], Y. Li[1,2], F. Zhang[1,2], M.W. Booze[1,2], E.R. Martin[1,2], W.K. Scott[1,2], J.L. Haines[2], M.A. Nance[2], J.P. Hubble[2], W.C. Koller[2], R. Pahwa[2], M. Stern[2], B.C. Hiner[2], J. Jankovic[2], C.G. Goetz[2], G.W. Small[2], F. Mastaglia[2], R.A. Gibson[2], L.T. Middleton[2], M.A. Pericak-Vance[1,2]*. 1) Duke Univ Medical Ctr, Durham, NC; 2) Duke CHG/GlaxoSmithKline PD Genetics Collaboration.

The pathological similarity between Alzheimer (AD) and Parkinson diseases (PD) has motivated many studies toward investigating the role of Apolipoprotein E (APOE) in PD. However, results have been inconsistent. We genotyped APOE functional polymorphism in 258 multiplex families (at least two affecteds per family). The aim of this study is to determine if APOE is associated with the risk or age at onset (AAO) of PD, or both. APOE allelic frequencies in our PD data are similar to those reported in AD, that is, APOE3 occurs the most frequent (71%) in the PD population. To test APOE for linkage and association with PD disease phenotype, PDT and TRANSMIT (TRM) programs were employed. APOE4 showed significant association to PD in both PDT and TRM (p=0.002 and 0.007, respectively), indicating that APOE4 is associated with PD susceptibility. We further examined the role of APOE in relationship to AAO in PD. The AAO in our PD dataset ranged from 12 to 90 years old. We treated AAO as a quantitative trait and employed the variance component approach implemented in QTDT program for the association analysis. The variance component approach models the mean and variance of the trait simultaneously, so that it is suitable for the data structure with multiple offspring per family. APOE2 showed evidence of association to AAO with p-value= 0.005. Furthermore, the average AAO of PD was later in patients with the 22/23 genotypes than in patients with 44 genotype (61±13.9 vs. 57±14.8), implying that APOE2 carriers have a later AAO than APOE4 carriers. We concluded that APOE gene is associated with the risk as well as AAO in PD. Specifically, APOE4 is a PD risk factor and APOE2 has a protective effect on delaying PD AAO.

Program Nr: 85 from 2002 ASHG Annual Meeting

**Modelling admixture to map genes underlying ethnic differences in phenotype.** *M.D. Shriver[1], C.J. Hoggart[2], P.M. McKeigue[2].* 1) Dept of Anthropolgy, Penn State University, University Park, PA; 2) Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, UK.

We describe a Bayesian model that quantifies the genetic basis for ethnic differences in phenotype and maps genes responsible for the ethnic differences. The method utilises marker genotype data from admixed populations at loci that exhibit large allele frequency differences between populations, ancestry informative markers (AIMs). The method estimates population and individual level admixture and loci ancestry. Linkage between loci is modelled. Any number of founding populations can be included and uncertainty in the allele frequencies of these founding population can be accounted for. We test the fit of the model allowing the detection of mis-specified population specific allele frequencies and comparisons of models with different numbers of founding populations. We map genes that underlie ethnic differences in phenotype by constructing score tests for linkage between ancestry at marker loci and phenotype.

We demonstrate the method using skin pigmentation as a model phenotype. 34 AIMs were genotyped in two population samples with primarily African ancestry, African Americans from Washington D.C. and an Afro-Caribbean sample from England, and 23 AIMs were genotyped in a sample of Mexicans from Colorado. The analyses of the three populations allowed for admixture between African, European and Native American ancestry. In the two African population samples we observed strong association between African ancestry and skin pigmentation (as measured by skin reflectometry). In the Mexican population we observed a strong association between Native American ancestry and skin pigmentation. The association confirms the validity of the method and also indicates the high level of population structure related to admixture. The distribution of admixture in the population is also available from the analysis. Our analyses showed that African ancestry at the TYR and OCA2 genes and Native American ancestry at the TYR and CYP genes are linked to skin pigmentation.

Program Nr: 86 from 2002 ASHG Annual Meeting

**Genome-wide microarray screening for genes harboring truncating mutations based on non-sense-mediated RNA decay and comparative genomic hybridization (NMD-CGH).** *P. Huusko[1], M. Wolf[1], J. Ruiz[1], M. Allinen[1], S. Hautaniemi[2], Y. Chen[1], S. Mousses[1], O. Kallioniemi[1]*. 1) NHGRI/NIH, Bethesda, MD; 2) Institute of Signal Processing/Tampere University of Technology, Tampere, Finland.

Tumor suppressor genes (TSGs) play a critical role in the pathogenesis of hereditary and sporadic cancer. According to the Knudsons two-hit hypothesis, cancer development involves a mutation in one allele and loss of the remaining wild type copy. Here, we developed a new strategy for genome-wide identification of genes that are inactivated in cancer. We first generated high-resolution maps of all deleted genomic regions in 7 breast and 4 prostate cancer cell lines by CGH analysis using an array of 15,000 cDNA clones. Deletion sites were visualized by a low moving average of copy number ratios (tumor vs. normal) for consecutive cDNA clones along the genomic sequence from 1pter to Yqter. We then applied the same cDNA microarray for screening of genes harboring truncating mutations based on blocking nonsense-mediated mRNA decay (NMD) by translation inhibiting drugs. The relative accumulation of transcripts before and after NMD inhibition, measured as a ratio in cancer vs. normal cells was used to rank genes most likely to harbor protein truncating mutations. For example, hMLH1 has a mutation in DU145 prostate cancer cell line and was ranked 16th of 15,000 genes (937X enrichment). p53 is mutated in PC3 prostate cancer cells and was ranked 436th of 15,000 (34X). A substantial improvement in sensitivity was obtained by overlaying NMD and CGH data on a gene by gene basis. For example, among the genes with elevated NMD ratios mapping to deleted genomic regions in the PC3 cells, p53 was ranked 25th (600X). Overall, bioinformatic analysis revealed 65 genes that were positive in the NMD assay and mapped to deleted regions in the corresponding samples. In summary, NMD-CGH is a powerful approach for genome-wide screening of genes that are likely to harbor truncating mutations in cancer. NMD-CGH should facilitate identification of novel TSGs in cancer as well as causative mutations in other genetic diseases.

Program Nr: 87 from 2002 ASHG Annual Meeting

**BRCA1 deficient cells have increased fragile site expression that is complemented by wild type BRCA1.** *M.F. Arlt[1], S.G. Durkin[1], A.M. Casper[1], M.B. Kastan[2], T.W. Glover[1].* 1) Dept Human Genetics, University of Michigan, Ann Arbor, MI; 2) Dept Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN.

Chromosomal common fragile sites form gaps or breaks when DNA synthesis is partially inhibited (e.g. with aphidicolin). Fragile sites are prone to rearrangement and frequently show large intralocus deletions in many tumor cells. Despite years of study, little is known about the mechanisms underlying fragility, or what cell cycle checkpoint/DNA repair pathways may be involved in the maintenance of genome integrity at those sites. We have shown that HCC1937, a breast cancer cell line deficient in BRCA1, expresses a greatly elevated level of total gaps and breaks at specific fragile sites when treated with 0.3 uM aphidicolin. In order to determine if the lack of BRCA1 is responsible for the increase in fragile site expression, we stably transfected HCC1937 cells with a BRCA1 expression construct. Treatment with aphidicolin revealed that the total gaps and breaks per cell dropped fourfold in untransfected cells, to near normal levels, in two independent clones. FISH analysis of FRA3B and FRA16D in these cells revealed a similar result. The number of gaps/breaks at FRA3B dropped three- to fourfold in the wild type transfected clones. The number of gaps/breaks at FRA16D dropped threefold in the transfected clones. HCC1937 transfected with empty vector showed no change in total gaps or breaks or expression of either fragile site. The same experiments were carried out in a mouse Brca1-/- cell line and matched controls. Again, the mouse cells expressing Brca1 showed a decrease in fragile site expression compared to the knockout line. These data indicate that BRCA1 is important in the proper maintenance of common fragile site sequences. Together with other results from our laboratory, these results suggest that fragile sites are recognized by the S-phase/G2-M checkpoint pathways in response to stalled replication forks. They further suggest that deletions or rearrangements at fragile sites in tumor cells could be a result of mutation or perturbations in these pathways.

Program Nr: 88 from 2002 ASHG Annual Meeting

**HoxA9 is a Novel Breast Cancer Progression Gene Identified by Microarray Analysis.** *M.A. Unger[1], J. Lakins[2], H.X. Zhang[1], W. Foster[4], B.J. Baxter[1], L. Chodosh[3], V.M. Weaver[2], B.L. Weber[1]*. 1) Cancer Genomics Program, University of Pennsylvania Cancer Center, Abramson Family Cancer Research Institute; 2) Pathology and Laboratory Medicine, Institute for Medicine and Engineering; 3) Departments of Molecular and Cellular Engineering and Endocrinology, University of Pennsylvania, Philadelphia, PA; 4) Bristol-Myers Squibb, Wilmington, DE.

This study was designed to identify and characterize novel progression genes with differential expression between normal breast tissue and invasive ductal carcinoma tissue from the same individual. Fresh frozen tissue was collected and macrodissected with a pathologist to identify populations of cells that were 85% or greater homogeneous normal breast tissue or invasive carcinoma. Five matched tumors and normal pairs have been examined using oligonucleotide microarrays. Extensive pathway mining identified HoxA9 as a novel progression target whose expression was significantly lower in the tumors compared to the matched normal adjacent tissue. RT-PCR confirmed a reduction in HoxA9 expression in 85% of breast cancers tested in a larger panel of normal and cancerous tissue and breast cancer cell lines. Heteroduplex mutation testing did not identify any variants in the splice sites or coding regions of HoxA9 in the samples tested for HoxA9 gene expression. However, bisulfite sequencing and treatment of MCF-7 cells with a demethylation reagent suggests expression of HoxA9 may be regulated at least in part, by methylation and possibly imprinting. Overexpression of HoxA9 in MDA-MB-231 cells led to the altered expression of a variety of genes, including some involved in cell cycle, cell adhesion and oncogenesis. Most interestingly, there was a dramatic increase in the expression of BRCA1 and MMP1 in cells overexpressing HoxA9. Further genomic analysis pinpointed Hox consensus sequences in the promoters of both genes. Finally, cell lines overexpressing HoxA9 had a 2.5 fold increase in motility compared to cell lines containing a vector only construct. These findings combined with previous studies suggest that HoxA9 may play a role in motility and branching morphogenesis perhaps through a pathway involving BRCA1 and MMP1.

Program Nr: 89 from 2002 ASHG Annual Meeting

**Genetic alterations in sentinel lymph node metastatic lesions compared to their corresponding primary breast tumors.** *L.R. Cavalli[1], C.A. Urban[2], S. de Assis[1], D.C. Tavares[3], J.D. Rone[1], R.S. Lima[2], I.J. Cavalli[4], B.R. Haddad[1]*. 1) Inst. for Mol. and Human Genetics, Lombardi Cancer Center, Georgetown University Medical Center, Washington DC, USA; 2) Departamento de Oncologia do Hospital Nossa Senhora das Graas, Curitiba, Pr, Brazil; 3) Universidade de Franca, SP, Brazil; 4) Departamento de Gentica, Universidade Federal do Paran, Curitiba, Pr, Brazil.

Accumulation of genetic aberrations is believed to play a pivotal role in each step of tumor development and progression. Studies comparing changes in the metastatic lesions with those found in the corresponding primary tumors, have revealed different genetic alterations between these lesions. In this study, we present the genetic changes detected by CGH in 6 paired primary breast tumors and their matched sentinel lymph nodes. The chromosomal alterations more frequently observed in the primary tumors were gains of 1p21-q24, 8q23-qter, 12q23-qter, 16p and 20q and in the sentinel lymph node group were: gains of 1p32-pter, 6p21-pter, 17 and 20 and losses of 2q22-q36 and 6q13-q23. Chromosomal alterations common to both groups included gains of 1q22-qter, 9q31-qter, 11p15-q21, and 20q and loss of 13q13-q32. This is the first study reporting the genetic alterations in the sentinel lymph node, the first node in the breast to harbor metastasis, compared to the corresponding primary tumors. Characterization of such genetic alterations in "early" metastatic lesions is of interest and may lead to the identification of the initial events associated with the metastatic dissemination process, and can be used in the future as an additional biological marker to help in the decision making on axillary lymph node dissection.

Program Nr: 90 from 2002 ASHG Annual Meeting

**Analysis of CHEK2 1100delC in North American breast cancer families and population based breast cancer series.** *T. Walsh[1], T-L. Young[1,5], S. Snook[1], B. Newman[2], B. Milikan[3], N.Y.B.C.S. Consortium[4], K. Laing[5], J. Green[5], M-C. King[1]*. 1) Medicine and Genome Sciences, University of Washington, Seattle; 2) Queensland University of Technology. Brisbane, Australia; 3) Lineberger Cancer Center, Univ North Carolina, Chapel Hill; 4) NYBCS Consortium; 5) Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland.

Mutations in BRCA1 and BRCA2 are highly penetrant and account for the majority of cases of hereditary breast and ovarian cancer. The remaining breast cancer susceptibility may be attributable to cryptic mutations in BRCA1 and BRCA2, as yet undiscovered additional highly penetrant breast cancer genes, or common alleles that confer lower risks than BRCA1 and BRCA2. It has recently been proposed that a truncating mutation in the cell cycle checkpoint kinase CHEK2 (1110delC) may be a low penetrance breast cancer allele increasing breast cancer risk two-fold in female carriers (Meijers-Heijboer et al., 2002). We have evaluated the relative risk this variant confers in two population based breast cancer studies with age and ethnically matched control samples. Both series confirm an approximate two-fold increased breast risk for CHEK2 1100delC carriers (RR 2.21). This variant also accounts for 2% (2/100) of high risk, BRCA1 and BRCA2 negative families from North America. In a second series of high risk breast cancer families from Newfoundland, the variant was also observed in 2% (3/144) of families. The CHEK2 1100delC variant was observed at a lower frequency (0.45% 4/905) among women with breast cancer and Jewish ancestry, negative for the three common Jewish BRCA1 and BRCA2 mutations. This allele was not observed in 173 African American breast cancer cases or 150 controls. Full sequencing of the CHEK2 gene at the cDNA and genomic DNA level in 48 probands from high risk breast cancer families did not identify any other additional deleterious alleles, suggesting that they maybe rare, cryptic or specific to other populations not screened in this study.

Program Nr: 91 from 2002 ASHG Annual Meeting

**Germline *PTEN* deletions cause a subset of classic Cowden and Bannayan-Riley-Ruvalcaba syndromes.** *X. Zhou, H. Hampel, M. Aulik, R. Pilarski, C. Eng.* Human Cancer Genetics, Ohio State Univ, Columbus, OH.

Cowden syndrome (CS [MIM158350]) and Bannayan-Riley-Ruvalcaba syndrome (BRRS [MIM153480]) are two autosomal dominant multiple hamartoma syndromes. CS is characterized by multiple hamartomas that affect derivatives of all three germ layers and by a high risk of breast, thyroid, and endometrial neoplasias. BRRS is characterized by macrocephaly, lipomatosis, hemangiomatosis and speckled penis. Germline mutations in *PTEN*, a tumor suppressor gene located on 10q23.3, have been found in 80% of probands with CS and 60% with BRRS, respectively. Routine PCR-based mutation detection techniques failed to detect a pathogenic germline *PTEN* mutation in 20% individuals with CS and 40% with BRRS. We sought to determine whether large deletions or rearrangements of *PTEN* are involved in and contribute to the phenotypes of CS and BRRS. We developed real time PCR assay to detect *PTEN* gene deletions by dosage. Germline DNA from 80 individuals with classic CS or BRRS, all *PTEN* mutation negative, were analyzed using this technique. Of these, 3 (3.8%) were found to have hemizygous *PTEN* deletions. Fine mapping of the 10q23 region using 8 dinucleotide markers and 2 *PTEN* intragenic polymorphic sites suggested that 2 of the 3 deletions encompassed the entire *PTEN* locus and the third deletion starts at least upstream of exon 5 and extends no further than intron 8. All 3 probands with deletions had prominent papillomatous papules, hemangiomas and multiple gastrointestinal tract hamartomatous polyps. These data suggest that hemizygous deletion of *PTEN* can result in CS or BRRS phenotypes but perhaps in 5% or less. These preliminary data suggest that looking for hemizygous deletions in PCR-based mutation negative CS or BRRS cases should beconsidered. If our observations that deletions are associated with severe papillomatous papules, hemangiomas and multiple hamartomatous polyps can be confirmed in a larger series, then deletion analysis in PCR-based mutation negative patients should be offered.

Program Nr: 92 from 2002 ASHG Annual Meeting

**The influence of genetic counseling and testing on uncertainty in a family at high risk for a *BRCA1* mutation.** *B.J. Baty, A.Y. Kinney, W.N. Dudley, E.K. Marshall.* Univ Utah Health Sciences Ctr, Salt Lake City, UT.

The commonly held notion that genetic testing reduces uncertainty has not been empirically tested. Based on anecdotal information, we predicted that genetic testing would decrease some types of uncertainty but increase other types of uncertainty, and that changes in uncertainty are associated with changes in anxiety. We tested our predictions in the Family Health Study, which offers genetic counseling and testing to adult men and women in a single African American kindred with over 30 cases of breast, ovarian, prostate and colon cancer, and an identified *BRCA1* mutation (1775T->G). Questionnaires were administered to study participants (62 women and 26 men) before genetic counseling and testing, and approximately 6 weeks after testing (25 women and 10 men to date). Participants rated questions regarding uncertainty about genetic testing and cancer risk reduction on a 1-5 scale with 1 indicating very uncertain and 5 indicating very certain. Anxiety was measured by the State Anxiety Inventory. Comparing the baseline responses to responses after counseling and/or testing, we found that participants did not significantly change their certainty about whether testing would have a positive effect on their lives. However, participants became more confident that they can detect cancer early (mean 3.1 vs. 3.8; $p < 0.01$), and more confident that they can cope well with genetic testing results (mean 3.5 vs. 4.4; $p < 0.01$). Participants also became more uncertain that testing will result in a negative effect on their lives (mean 2.6 vs. 1.8; $p < 0.01$), and experienced a significant decrease in anxiety ($p < 0.05$). In addition, participants who decreased in certainty about negative effects had a significant reduction in anxiety ($p < 0.05$), while those who increased in certainty about negative outcomes did not manifest an appreciable change in anxiety. Because the effect of genetic testing on uncertainty has not been empirically examined previously, these results cannot be compared to other studies. However, preliminary results indicate a complex interplay among measures of uncertainty and anxiety, and point to the need for further study.

Program Nr: 93 from 2002 ASHG Annual Meeting

**Patents and monopolies on diagnostic tests: Europe's opposition against the BRCA1 patents.** *G. Matthijs[1], D. Halley[1], K. Sperling[1], S. Kølvraa[1], R. Elles[1], D. Stoppa-Lyonnet[2].* 1) For the Belgian, Dutch, German, Danish and British Societies for Human Genetics, resp; 2) for Institut Curie, France.

In 2001, the European Patent Organisation (EPO) has granted 3 patents on the BRCA1 gene (EP-B-699754, EP-B-705903, EP-B-705902) to Myriad Genetics. The patent system is there to protect intellectual property (IP) and allow inventors a financial return. Society benefits from products arising from the invention. The approach taken by the holders of the BRCA1 patents challenges the 'contract' between inventors and society. The case illustrates the risks of the monopoly given by 'ownership' of a gene sequence when patent holders refuse to license their invention on reasonable terms. This development has evoked a strong reaction in Europe.

The European Patent Convention (EPC) allows a democratic control on patenting via an opposition procedure. In parallel, a French association of research institutes and hospitals, and a coalition of the Belgian, Dutch, British, Danish and German genetic societies have opposed EP-B-699754. The same group - now also including the Deutsche Krebshilfe - and the Belgian and Dutch governments have opposed EP-B-705903. The deadline for EP-B-705902 is 28-8-2002. The opponents attack the patents on the priority date and on errors contained in the original sequences. There is a lack of inventive step: the patentees have benefited strongly from research through an international consortium on BRCA with the collaboration of patients and families. Moreover, many hold that the relationship between mutations and a disease constitutes a discovery not an invention. Also, EPC excludes diagnostic methods practised on the human body from patenting; the opponents argue that mutation analysis of a disease-related gene using a blood sample constitutes a medical act within the meaning of Art. 52(4).

The opponents call upon EPO to revise the current interpretation and urge the patent owners to reconsider their approach to exploiting gene sequence IP. They call upon legislators, industries and the public to also consider the issues surrounding licensing DNA sequences.

Program Nr: 94 from 2002 ASHG Annual Meeting

**Multiple breast and/or ovarian cancers as covariates in a hereditary prostate cancer genomic scan confirmations the 16q23 locus and implicates it in multiple cancer types.** *G.P. Jarvik[1], M.D. Badzioch[1], M. Janer[2], M.A. Peters[3], S. Kolb[3], R. Zaucha[3], E.L. Goode[1], D. Fredrichsen[3], H.B. DeFrance[3], L. Hood[2], E.A. Ostrander[3], J.L. Stanford[3]*. 1) Div Medical Genetics, Univ Washington Medical Ctr, Seattle, WA; 2) Institute for Systems Biology, Seattle, WA; 3) Fred Hutchinson Cancer Research Center, Seattle, WA.

Hereditary prostate cancer (HPC) is a genetically heterogeneous disease, which complicates the efforts to map and clone susceptibility loci. Consideration of primary cancers at multiple sites has helped define locus heterogeneity in other familial cancer disorders by allowing stratification into more homogeneous subgroups. There is an excess of breast cancer in HPC families and breast and ovarian cancer co-occurrence within families was high, thus, we used breast and ovarian cancers as covariates to help identify HPC loci in a 10CM genomic scan. We evaluated a set of 94 families, 13 of which had multiple cases of breast cancer and/or ovarian cancer in first-degree relatives of men with prostate cancer. BRCA 1 or 2 mutations were not detectable. The 13 families contained 91 affected males (65 sampled) with an average median age at diagnosis of 65.3 yrs. There were 33 cases of breast and 11 of ovarian cancer in first-degree relatives of affected men. A region on chrom. 16q23 gave a lod score of 2.38 at marker D16S2624 and a maximized lod (mlod) score of 3.66 and multipoint lod score of 4.22 under higher penetrance assumptions in these 13 families. An mlod >3.3 is considered significant. Additionally, a regression method linkage analysis found that the breast-ovarian strata used as a covariate significantly predicted a linked subset ($p<.01$) using D16S2624. This region is commonly deleted in prostate and breast cancer tumors and has previously given significant evidence of linkage to HPC. These results confirm HPC linkage to the 16q23 region. Additionally, these results suggest this locus may harbor a tumor suppressor gene that influences multiple cancer types and that co-occurrence of multiple breast and/or ovarian cancers with prostate cancer may be a useful indicator of HPC families linked to 16q23.

Program Nr: 95 from 2002 ASHG Annual Meeting

**Validation of the GREAT system for automated collection of the cancer pedigree.** *L.S. Acheson[1], K.C. Stange[1], S.J. Zyzanski[1], G.L. Wiesner[2].* 1) Dept Family Medicine, Case Western Reserve Univ, Cleveland, OH; 2) Depts of Genetics and Medicine, Case Western Reserve Univ and Center for Human Genetics, University Hospitals, Cleveland, OH.

The GREAT (Genetic Risk Easy Assessment Tool) enables patients to call a computer-assisted telephone interview to record a detailed family history of cancer and immediately generates a pedigree in digital form. Purpose: To validate the family cancer histories produced via the GREAT by comparison with pedigrees made by genetic counselors. Methods: Patients scheduled for genetics consultation were eligible to record their family histories using the GREAT, separate from their genetic counseling session. The number and age of each relative; presence, type, and age at diagnosis of cancers; and cancer geneticist's assessment of familial cancer risk were compared for 120 pairs of pedigrees produced by counselors and GREAT. Results: The telephone interview took a mean of 33.5 minutes and was highly acceptable to respondents. 95% of first degree relatives, 68% of second degree relatives, and 38% of third degree relatives were identical on pedigrees from counselors and the GREAT. GREAT pedigrees included additional second and third degree relatives not shown on counselors' pedigrees. Sixty-three percent of all cancers were identified by both counselor and GREAT, with 90% agreement on type of cancer; 21% of cancers were identified only by the GREAT and 16% only by the genetic counselor. There was very good agreement (kappa=.72, correlation=.78) between the geneticist's breast cancer risk assessments based on the GREAT vs. counselors' pedigrees. Analyzing a subgroup of patients who came for cancer genetics consultation revealed closer agreement between the GREAT and genetic counselors: 71% of all cancers were identified by both, 11% only by the GREAT, and 18% only by the counselor. Conclusions: The GREAT interactive telephone system is a valid method for collecting family histories of cancer, as demonstrated by comparison with pedigrees obtained by genetic counselors during genetic consultations. This automated process for generating pedigree data can potentially save time in clinical and research settings.

Program Nr: 96 from 2002 ASHG Annual Meeting

**An Alu Transposition Model for the Origin of Human Segmental Duplications.** *J.A. Bailey, E.E. Eichler.*
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Recent segmental duplications have been shown to play pivotal roles in the evolution and pathology of the human genome. Compared to other model organisms (Drosophila and Celegans), the human genome is unique in the relative proportion of such duplications. The molecular basis for this difference is unknown. While it is likely that multiple mechanisms are responsible for this architecture in the genome, considerable insight into the mechanisms underlying duplication and transposition can often be obtained by studying the junctions or transition regions of such events. We sought to determine if certain sequence signatures were enriched at the boundaries (or junctions) of duplications. We performed a global analysis wherein the sequence content of the 50 bp encompassing each pairwise junction to control sequence in terms of high copy repeat density as well as GC content. The control region was comprised of the entire duplication along with 1 kb flanking sequence on either side. To minimize multiple rearrangement events, we limited our comparisons to large highly similar duplications (95-99.5% and >5kb). We analyzed a total of 550 junctions comprising 1% of the human genome. The only significant difference between junction and control sequence was an increase in the presence of Alu elements (29.2% vs. 16.3%; $P < 0.001$). When we classified Alu elements into major families, with respect to evolutionary age, there was a gradient of enrichment in terms of elements age, with the youngest Alu elements (AluY) having the greatest enrichment. The oldest Alu elements show no appreciable enrichment. Combined this data suggests that Alu elements may mediate a significant fraction of the excision and/or integration events giving rise to segmental duplications within the human genome. This association may help to explain the preponderance of such events in the human lineage of evolution.

Program Nr: 97 from 2002 ASHG Annual Meeting

**Characterization of low copy repeats on 22q11 (LCR22) that mediate chromosome rearrangement disorders.** *M. Babcock, I. Ioshikhes, Z. Li, B.E. Morrow.* Molecular Genetics, Albert Einstein College of Med, Bronx, NY.

The chromosome 22q11 region is susceptible to rearrangements leading to velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS), der(22) syndrome and cat-eye syndrome (CES), mediated by low copy repeats on chromosome 22q11 (LCR22). To understand the molecular basis of chromosome rearrangements, we defined the architectural features of the LCR22s. Two of the LCR22s, LCR22-2 and LCR22-4, are 200 kb in size and mediate the common 3 Mb VCFS/DGS deletion and CES duplication. One of the LCR22s (LCR22-3) has not been completely mapped in humans due to the lack of bacterial clones that span it. The rest of the LCR22s are much smaller in size and together, the seven comprise 600 kb of sequence. We examined the LCR22s to determine whether full-length genes are present. At least seven different genes map to the LCR22s and they are USP18, XM_092877/78 (predicted), E2F6 (pseudogene), KIAA1292 (predicted), BCR, GGTLA and GGT1. The USP18, KIAA1292, BCR and GGTLA1 genes impinge on LCR22-2, LCR22-3, LCR22-6 and LCR22-7, respectively while the GGT1 is present within LCR22-8. In addition, all the LCR22s contain duplicated copies of exons derived from each of the genes. The fact that both full-length and partial gene copies are dispersed among the LCR22s suggests that they have formed by duplication and recombination processes during evolution. To determine whether the LCR22s show continued evidence for such processes, we examined the sequence for LCR22 specific nucleotide alterations (cismorphisms). There were a surprisingly few cismorphisms between LCR22-2 and LCR22-4 as compared with the more telomeric ones suggesting either that they have evolved more recently or that gene conversion has occurred. As compared to the human situation, the mouse does not have LCR22s but possesses single copies of the genes. Of interest, some of them are near the evolutionary breakpoints between the mouse and human genomes. We believe that understanding the basis of instability in the LCR22s will provide insights into mechanisms of chromosome rearrangements leading to genomic disorders.

Program Nr: 98 from 2002 ASHG Annual Meeting

**ATR is responsible for genome stability at fragile sites.** *A.M. Casper[1], P. Nghiem[2], M.F. Arlt[1], T.W. Glover[1]*. 1) Dept. of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Dept. of Chemistry and Chemical Biology, Howard Hughes Medical Institute, Harvard University, Cambridge, MA.

Agents that partially inhibit DNA replication have long been known to induce expression of common fragile sites. These sites are frequently deleted and rearranged in many tumor types. Yet, the mechanisms of fragile site expression have been elusive. Recent studies have indicated that the ATM (Ataxia Telangiectasia [AT] Mutated) and ATR (AT and Rad3 Related) genes are central to the S and G2/M checkpoints. Based on the instability of fragile sites and the possible role of replication fork stalling at these sites, we investigated the role of ATM and ATR in fragile site stability. ATM is known to respond primarily to double strand breaks. ATR is more difficult to study, as it is required for cellular viability. However, ATR has very recently been shown to act in parallel with ATM and to respond primarily to stalled replication forks. The kinase function of both proteins is inhibited by caffeine and by 2-aminopurine. We have demonstrated that 2-aminopurine, like caffeine, increases fragile site expression. Using AT cell lines, we found no effect of ATM deficiency on fragile site expression. We used three approaches to overcome difficulties in studying ATR: cells stably transfected with a dox-inducible dominant negative ATR construct, RNAi against ATR and cre-lox mediated ATR deletion. Cells with ATR deficiency by all three approaches showed a four- to ten-fold increase in breaks and gaps at specific fragile sites as compared to control cells. Our results clearly demonstrate that ATR is critical for fragile site stability. Thus, tumor cells with mutations in the ATR checkpoint pathway are predicted to show deletions and rearrangements at fragile sites. This is the first major molecular pathway to be associated with genome stability at fragile sites. The function of ATR provides a basis for understanding the mechanism of fragile site instability and, together with other results from our laboratory, allows us to propose a model for fragile sites based on unreplicated regions caused by stalled or collapsed replication forks recognized by the ATR/BRCA1 checkpoint pathway.

Program Nr: 99 from 2002 ASHG Annual Meeting

**Protein kinase A may be involved in chromosomal stability: Induction of cytogenetic abnormalities in mouse embryonic fibroblasts by PRKAR1A down- regulation.** *L.D. Matyakhina[1], S. Lenherr[1], F. Sandrini[1], L.S. Kurschner[1], A. Dutra[2], E. Pak[2], C.A. Stratakis[1]*. 1) Dept DEB, NICHD, Bethesda, MD; 2) Cytogenetic & Confocal Microscopy & Confocal Core, NHGRI, Bethesda, MD.

Carney complex (CNC) is an autosomal dominant multiple neoplasia syndrome. Cytogenetic rearrangements has been reported in cultured cells from tumors of CNC patients. Inactivating mutations in the tumor suppressor gene *PRKAR1A* were identified in families mapping to 17q22-24 and in several sporadic patients. In these cases, no mutant PRKAR1A protein is made; along with loss-of-heterozygosity (LOH) of the normal allele in CNC tumors, this finding suggested that in affected tissues no PRKAR1A protein is produced. We hypothesized that down regulation of *PRKAR1A* would be responsible for the genomic instability observed in CNC tumors. Stably transfected mouse embryonic fibroblasts (MEF) cell lines expressing a regulatable antisense (as)-PRKAR1A under the control of a tetracycline-responsible promoter were generated. Free protein kinase A (PKA) activity and PRKAR1A protein amount were determined in two clones (X21 and X22) of these cell lines by a standard PKA assay and western blotting, respectively. Cytogenetic examination of the X21 and X22 clones was obtained on passages 2 and 10 and compared with control MEF cells. Both cell lines showed lower free PKA and PRKAR1A protein in their AS-on vs. AS-off state, albeit X21 PRKAR1A protein downregulation was less than that in X22. Cytogenetic analysis data correlated with the level of as-PRKAR1A expression in the two clones, most data being significant only in X22. In X22, the total number of cytogenetic changes was higher in the AS-on state in p10 ($p<0.00001$) but not in p2 ($p=0.445$). However, two changes, in particular, dicentric chromosome formation and translocations were significantly more frequent in both p2 and p10 in the AS-on state ($p<0.001$). We conclude that down- regulation of *PRKAR1A* in MEF cells results in a greater number of certain cytogenetic rearrangements, namely dicentric chromosomes and, to a lesser degree, translocations. This is the first in vitro evidence of the PKA enzyme being involved in the preservation of chromosomal stability.

Program Nr: 100 from 2002 ASHG Annual Meeting

**The Bloom syndrome helicase BLM interacts with the telomeric protein TRF2 and promotes amplification of telomeric DNA in telomerase-negative immortalized cells.** *M.S. Meyn[1,2], P. Bradshaw[1,2], X. Li[1], K. Truong[3], I. Pasic[1,2], M. Ungrin[3], M. Ikura[3], D.J. Stavropoulos[1,2].* 1) Genetics, Hosp. for Sick Children, Toronto, ON; 2) Molec. & Medical Genetics, Univ. of Toronto, Toronto, ON; 3) Medical Biophysics, Univ. of Toronto, Toronto, ON.

The *BLM* gene codes for a recQ helicase and is mutated in Bloom syndrome, a recessive condition marked by growth retardation, immunodeficiency, cancer and genetic instability. ~10% of human tumors lack telomerase and maintain their telomeres by Alternative Lengthening of Telomeres (ALT) pathways. ALT may involve genetic recombination, but the proteins required for ALT have not been identified. We now report data implicating BLM in the ALT maintenance of telomeres.

We found BLM forms multiple nuclear foci in ALT human fibroblasts. A majority of BLM foci co-localized with telomeric foci in ~70% of interphase cells. This behavior was ALT-specific, as BLM and telomeric foci rarely co-localized in telomerase-positive or primary cells. We used fluorescence resonance energy transfer (FRET) to test for in vivo interactions between BLM and the telomeric protein TRF2. Using ALT cells expressing BLM fused to CFP (CFP-BLM) and TRF2 fused to YFP (YFP-TRF2) we found FRET in ~10% of cells that showed co-localization between YFP-TRF2 and CFP-BLM. The FRET data indicated <10 nm separates YFP-TRF2 and CFP-BLM molecules. BLM and TRF2 interactions were confirmed by co-immunoprecipitation. Over-expression of GFP-BLM resulted in rapid, ALT-specific amplification of telomeric DNA, as determined by FLOW-FISH and interphase FISH. 60 hours post-transfection, fluorescence intensities of individual telomeric DNA foci in GFP-BLM expressing cells were often more than 10-fold greater than the brightest foci in controls, supporting a model for ALT in which telomeres are maintained through rolling circle amplification rather than break-induced replication.

Our results identify BLM as the first protein found to affect ALT-specific telomeric DNA synthesis, imply that TRF2 and BLM directly interact at a subset of ALT telomeres and suggest that BLM facilitates recombination-driven amplification of telomeres in ALT cells.

Program Nr: 101 from 2002 ASHG Annual Meeting

**Deletion of the *Drosophila* homologue of MEN1 causes hypersensitivity to ionizing radiation.** *V. Busygina, K. Suphapeetiporn, A.E. Bale.* Dept Genetics, Yale Univ Sch Medicine, New Haven, CT.

Multiple Endocrine Neoplasia type 1 (MEN1) is an autosomal dominant cancer syndrome affecting primarily the pituitary, parathyroids and pancreatic islets. MEN1 encodes a nuclear protein, menin, which has no homology to any other known protein and no recognized functional motifs besides nuclear localization signals. LOH studies suggest that MEN1 is a tumor suppressor, but analysis of the protein in mammals has offered little insight into the mechanism of carcinogenesis.

The *Drosophila* homologue of MEN1 (Mnn1) shares 46% identity and 57% similarity with human menin. There are no existing loss-of-function mutants of Mnn1 that would help assess the normal function of the gene. We mobilized a P-element (transposable element), located 5' to Mnn1, in the male germline to generate a 9.5 kb deletion that removed the promoter region and 50% of the Mnn1 coding region. Flies carrying the Mnn1 mutant allele on one chromosome and a deficiency covering the Mnn1 region on the other were viable with no visible morphologic abnormalities. Their viability was slightly lower than Mnn1 heterozygotes or WT flies. Mnn1 mutants also had delayed development (12 days instead of the normal 10). Fertility was normal in females, but males were infertile and had aberrant mating behavior. In searching for conditional phenotypes, we found that flies lacking Mnn1 were hypersensitive to ionizing radiation. Mutant, heterozygous, and wild type larvae were irradiated with 0, 1, 2, and 2.5 KRads and survival to adulthood was evaluated. Mnn1 mutants exhibited a 5-10 fold decrease in survival at 2.5 KRads. We are currently investigating G2/M DNA damage checkpoint and double strand break repair in these mutants. We are also generating point mutations of Mnn1 and using an Mnn1 rescue construct to confirm that the phenotypes described here are attributable to Mnn1 alone.

Our data suggest that MEN1 plays a role in sensing or repairing DNA damage. MEN1 may be a gene involved in maintenance of genomic integrity rather than a classical tumor suppressor.

Program Nr: 102 from 2002 ASHG Annual Meeting

**Effects of interruptions on microsatellite mutation rates in cultured cells.** *J.C. Boyer, L. Stefanovic, G.A. Smith, A. Castro, R.A. Farber.* University of North Carolina at Chapel Hill.

The results of several types of studies have suggested that the extent of microsatellite instability is correlated with the length of the repeat and that interruptions in the sequence result in increased stability. Alleles at trinucleotide repeat loci associated with human disease that contain interruptions are less susceptible to expansion than alleles with pure repeat tracts. In yeast, imperfections reduce microsatellite mutation rates by 5-90X, depending upon the type of repeat. We have quantitated the effects of sequence interruptions on the mutation rates of mononucleotide and dinucleotide repeats in cultured mammalian cells. Plasmids containing repeats of A, G, and CA of various lengths, with and without interruptions, were introduced into mismatch repair proficient ($MMR^+$) human and mouse fibroblasts and $MMR^-$ human colorectal cancer cells. The plasmid contained a microsatellite that was inserted near the 5' end of a neomycin resistance gene (neo), such that the reading frame of the neo gene was disrupted. Clones with frameshift mutations in the microsatellite sequence that restored the reading frame were selected in the neomycin analogue G418, and mutation rates were determined by fluctuation analysis. In general, higher mutation rates were observed in longer microsatellites, and interruptions stabilized some long repeats but the magnitude of the stabilization was dependent upon the repeat composition and MMR status of the cells. The $A_{17}$ repeat was stabilized 20-60X by a single-base interruption and the stabilization was independent of MMR. A single-base interruption did not stabilize $G_{17}$ in $MMR^-$ cells but did stabilize $G_{17}$ in $MMR^+$ cells by a factor of 75X. The CA repeat was not stabilized by an interrupting TA; however, preliminary data suggest that a TG interruption lowers the mutation rate. Among mononucleotide repeats, $G_{17}$ repeats were more unstable than $A_{17}$ repeats. This was independent of MMR, suggesting that replication fidelity of $G_{17}$ is lower than that of $A_{17}$. Mutation spectra suggest that 2-bp frameshift loops in the template strand are more efficiently repaired than 1 or 4-bp loops in the primer strand.

Program Nr: 103 from 2002 ASHG Annual Meeting

**Mismatch repair gene *Msh2* is a genetic modifier of CAG repeat instability and striatal-specific disease in *HdhQ111* knock-in mice.** *V.C. Wheeler[1], L.-A. Lebel[1], V. Vrbanac[1], H. te Riele[2], M.E. MacDonald[1].* 1) Molecular Neurogenetics Unit, Massachusetts General Hospital, Charlestown, MA; 2) Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Huntingtons disease (HD) is a dominant neurodegenerative disorder caused by the expansion of a polymorphic CAG repeat that elongates a glutamine tract in huntingtin. The mutant version of this novel protein triggers the demise of vulnerable medium spiny neurons in the striatum and the onset of motor symptoms. The expanded *HD* CAG repeats exhibit striking intergenerational instability, as well as somatic instability with longer repeat lengths. Although the underlying process that determines disease onset is strongly CAG length-dependent, modifying factors have been implicated by variability in age at onset not accounted for by CAG size.

Studies of the *HD* mutation in *Hdh* knock-in mice, accurate genetic models of the disease, have shown that long unstable CAG repeats that lengthen the glutamine tract in murine huntingtin initiate a striatal-specific disease process. We have tested *Msh2* as a potential modifier of the *HD* defect, by assessing the impact of Msh2 deficiency on CAG instability and on an early striatal disease phenotype in progeny of *HdhQ111* and *Msh2* knock-out mice. Our results implicate multiple pathways in *Hdh* CAG instability; while maternally inherited changes, and paternally derived contractions were Msh2-independent, Msh2 deficiency eliminated repeat expansions in the male germline and adult striatum. Remarkably, Msh2 deficiency was found to influence the onset of striatal disease, with nuclear huntingtin accumulation delayed by more than 5 months. Immunoblot analysis of mutant huntingtin in the presence and absence of Msh2 suggests that Msh2 deficiency may slow disease by reducing mutant huntingtin molecules with somatic expansions of the glutamine repeat. Thus, our results provide strong support for the investigation of Msh2 as a genetic modifier of the behavior and consequences of the *HD* defect in man.

Program Nr: 104 from 2002 ASHG Annual Meeting

**Mutational mechanisms of Williams-Beuren syndrome deletions.** *L.A. Pérez-Jurado, L.F. Magano, R. Flores, M. Bayés.* Unitat de Genètica, Universitat Pompeu Fabra, Barcelona, Spain.

Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder that results from a recurrent heterozygous deletion of contiguous genes at 7q11.23. Three large region-specific low-copy repeat elements (LCRs) composed of different blocks (called A, B and C) flank the WBS deletion interval and are thought to mediate the *de novo* rearrangements that occur at a rate close to $2.5x10^{-5}$/gamete/generation. In this study, we have determined the exact deletion size and LCR copy number in 74 patients with WBS, as well as precisely defined deletion breakpoints in 32 of them using site-specific nucleotide differences. Most patients (95%) exhibit a 1,52 Mb deletion caused by recombination between centromeric and medial block B copies, which share ~99.6% sequence identity along 105 - 143 kb. Deletion breakpoints were mapped at several sites within the recombinant block B, with a cluster (35-55%) occurring at an 11 kb region within the *GTF2I/GTF2IP1* gene. Heterozygosity for genomic polymorphism was found in more than one-third of transmitting progenitors, including 1.8-2.4 Mb inversions between centromeric and telomeric LCRs (30%) and aberrant number of LCR blocks (4.5%). All deletion breakpoints in the patients with the inversion occurred in the distal 38 kb regions only present in the telomeric and medial block B copies. Finally, only 4 patients (5%) displayed a larger deletion (~1.67 Mb) caused by recombination between centromeric and medial block A copies. Haplotype data demonstrated that WBS deletions result from unequal interchromosomal recombination during meiosis I in all cases bearing the inversion, while both intra- and interchromosomal events are found in the absence of inversions. We propose models for the specific pairing and precise aberrant recombination leading to each of the different germline rearrangements that occur in this region, including inversions, deletions or duplications of LCRs, and deletions associated with WBS. Genomic instability at 7q11.23 is directly related to the genomic structure of the region.

Program Nr: 105 from 2002 ASHG Annual Meeting

**Evolutionary history and promoter characteristics interact to determine susceptibility to X chromosome inactivation.** *A.M. Friel[1], K.D. Tsuchiya[2], I.P. Ioshikhes[1], M. Fazzari[1], J.M. Greally[1]*. 1) Medicine/Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Vanderbilt-Ingram Cancer Center, Nashville, TN.

It has been estimated that one-fifth of the genes on the human X chromosome escape X inactivation. We have previously shown that the genomic context of imprinted promoters is distinctive in terms of its transposable element (TE) content. TEs are unusually distributed on the X chromosome, with more L1 LINEs and fewer Alu SINEs than autosomes. We explored whether local fluctuations in TE content or other variables explained the escape of a subset of genes from X inactivation. We found that the critical variables distinguishing inactivating (n=41), escaping (n=25) and autosomal (n=100) loci were promoter characteristics and evolutionary age of the chromosomal region, and that the 100 kb genomic context does not distinguish these loci in the same manner as imprinted loci. Distinctive promoter characteristics included a significantly higher proportion of CpG islands at inactivating loci compared with the other samples. The 4 kb context of the transcription start sites were analysed for transcription factor binding sites, with PAX-4 significantly over- and YY1 and OCT1 under-represented at inactivating promoters. A WORDUP analysis of random hexamers in this window defined novel (predominantly CpG-containing) motifs that are over-represented at the inactivating loci. The evolutionary ages of different strata within the human X have previously been defined. We found that the younger strata have not only quantitative but also qualitative differences in L1 LINE content, being truncated at their 5' ends in the younger evolutionary strata. As L1 LINEs are believed to be candidates for spreading the X inactivation signal, the preservation of 5' sequences in the strata in which inactivation is more uniform focuses attention on this component of the L1 as the specific mediator of inactivation spreading. X inactivation emerges as the outcome of an interplay between the large chromosomal subregions defined by the evolutionary strata and the promoters of the genes contained within them.

Program Nr: 106 from 2002 ASHG Annual Meeting

**Adult Psychiatric Phenotype of 22q11.2 Deletion Syndrome.** *A.S. Bassett[1,2], E. Chow[1,2], M. Gheorghiu[2], P. AbdelMalik[2], R. Weksberg[3,4].* 1) Dept Psychiatry, University of Toronto, Toronto, ON, Canada; 2) Clinical Genetics Research Program, CAMH,Toronto, ON, Canada; 3) Dept Paediatrics, University of Toronto, ON, Canada; 4) Clinical and Metabolic Genetics, HSC, Toronto, ON, Canada.

Background: 22q11.2 Deletion Syndrome (22qDS) has a variable phenotype including prominent neurobehavioural manifestations. Objective: To understand the range of psychiatric disorders and symptoms in adults with 22qDS. Method: Standard psychiatric assessments were used to assess DSM-IV diagnoses and severity of psychiatric symptoms in 46 adults with 22qDS ascertained from different sources. Results: In a subset ascertained for Tetralogy of Fallot (n=22), at a mean age of 26 years, 27% had schizophrenia, 18% anxiety disorders, 18% major depression, 45% had no history of major psychiatric illness. Comparing 21 non-psychotic (NP) 22qDS subjects with 44 siblings of individuals with familial schizophrenia (FS) who are also at elevated genetic risk for schizophrenia, the 22qDS-NP group had a higher rate of anxiety disorders (35.3% vs. 6.8%, p=0.008) but similar rates of major depression. The 22qDS-NP group also had significantly greater negative symptom (p=0.01) and excitement symptom severity (p=0.05). Comparing 22qDS-SZ with FS, there were no significant differences in age at onset (mean age 21), functioning, or four major symptom domains. However, impulsivity and hostility were more severe in 22qDS-SZ. Conclusion: The rate of schizophrenia in 22qDS is consistently found to be 25-30%, similar to the rate of congenital heart defects (CHD) in 22qDS samples not ascertained for CHD. Anxiety disorders are also common. Treatable psychiatric disorders are important later onset features of 22qDS, requiring consideration in genetic counselling.

Program Nr: 107 from 2002 ASHG Annual Meeting

**Characterization of a progressive neurological condition in older adult male carriers of the fragile X premutation.** *S. Jacquemont[1], R.J. Hagerman[1], M. Leehey[2], C. Greco[3], J. Brunberg[4], F. Tassone[5], L.W. Gane[1], T. Jardini[1], S.W. Harris[1], L. Zhang[6], J. Grigsby[7], V. Des Portes[1], E. Berry-Kravis[8], W.T. Brown[9], P.J. Hagerman[5]*. 1) M.I.N.D. Institute, UC Davis Medical Center, Sacramento, CA; 2) Dept. of Neurology, UCHSC, Denver, CO; 3) Dept. of Pathology, UC Davis Medical Center, Sacramento, CA; 4) Dept. of Radiology, University of California, Davis, CA; 5) Dept. of Biological Chemistry, University of Califnoria, Davis, CA; 6) Dept. of Neurology, UC Davis Medical Center, Sacramento, CA; 7) Dept. of Medicine, UCHSC, Denver, CO; 8) Dept. of Pediatrics, Rush-Presbyterian & St. Luke's Medical Center, Chicago, IL; 9) New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY.

We present a series of 20 male carriers of the fragile X premutation over 50 years of age who are affected by a multi-system, progressive neurological disorder. The principal clinical features of this syndrome include ataxia, intention tremor, short-term memory loss, executive function deficits and cognitive decline. Additionally, more variable features include Parkinsonism, peripheral neuropathy, lower limb proximal muscle weakness and autonomic dysfunction. The neuroradiologic findings are consistent and include moderate to severe generalized atrophy and hyperintensity of the middle cerebellar peduncles. These hyperintensities may be related to spongioform changes observed in the same region on neuropathological studies in two patients. Molecular findings include elevated mRNA and mildly decreased levels of FMRP. Preliminary epidemiological data will also be presented regarding a survey of 80 carriers greater than 50 years of age in California. 20% of male premutation carriers have this syndrome with radiological findings; 0% of female carriers, 0% of female controls, and 6% of male controls have tremor. These preliminary data suggest that 20% of older male carriers may develop this syndrome; however, there is likely ascertainment bias for affectedness in these initial results.

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Program Nr: 108 from 2002 ASHG Annual Meeting

**Genetic Linkage of Attention Deficit Hyperactivity Disorder to a region on Chromosome 16p13.** *V. Kustanovich[1], S. Minassian[2,3], J. Stone[1], M. Ogdie[1], J.J. McGough[4], J.T. McCracken[4], I.L. MacPhie[5], S.E. Fisher[5], R.M. Cantor[1], A.P. Monaco[5], S.F. Nelson[1,3].* 1) Department of Human Genetics, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Biostatistics, Univ California, Los Angeles, Los Angeles, CA; 3) Center for Neurobehavioral Genetics, Univ California, Los Angeles, Los Angeles, CA; 4) Department of Psychiatry and Biobehavioral Sciences, Univ California, Los Angeles, Los Angeles, CA; 5) Wellcome Trust Centre for Human Genetics, Oxford, U.K.

Attention deficit hyperactivity disorder is a common neurobehavioral disorder with early-onset in which impulsive, hyperactive, or inattentive behaviors lead to impairment in school, home, or social functioning (American Psychiatric Association, 1994). A recently completed genome scan for ADHD susceptibility genes (Fisher et al., 2002) has uncovered several suggestive regions although none showing significant genetic linkage with ADHD. In order to test the significance of suggestive linkage on 16p, identified in a genome scan of attention deficit hyperactivity disorder (ADHD), we performed fine mapping with a set of 11 microsatellite and 5 SNP markers from 18-40cM from the 16ptel. Genetic markers were genotyped by high throughput capillary electrophoresis (for the microsatellite markers), fluorescence polarization and single base extension and hybridization capture on a tag array (for the SNPs). Affected sib pair analysis (ASP) of 277 ASPs in 203 families was done using ASPEX 2.2. Here we report significant genetic linkage to a region on chromosome 16p which overlaps with several previously published susceptibility loci in Autism. We found strongest linkage to marker D16S3114 (MLS = 4.2) with the 1 LOD support interval extending ~7 Mb. This finding represents the most significant linkage evidence for a susceptibility gene for ADHD.

Program Nr: 109 from 2002 ASHG Annual Meeting

**Low adaptive behavior and cognitive functioning in patients with Smith-Magenis syndrome [del(17)(p11.2p11.2)].** *N.S. Madduri[1,4], M. Turcich[1,4], J.R. Lupski[2,3,4,5], L. Potocki[3,4,5]*. 1) Meyer Center for Developmental Pediatrics; 2) Department of Pediatrics; 3) Department of Molecular and Human Genetics; 4) Texas Children's Hospital; 5) Baylor College of Medicine.

Smith Magenis syndrome (SMS) is a multiple congenital anomalies and mental retardation syndrome associated with a deletion of chromosome 17 [del(17)(p11.2p11.2)]. Neurobehavioral abnormalities include sleep disturbances, EEG abnormalities and seizures, and aggressive and self-injurious behavior. Fifty-five patients with SMS (10 months to 31 years) were evaluated as part of a multidisciplinary clinical protocol at the Texas Children's Hospital. Behavior problems included aggressive actions, biting, hitting, self-injury, and were reported by parents to occur in 38% (N=21/55). Neurodevelopmental assessments done by a developmental pediatrician consisted of a Clinical Adaptive Test/Clinical Linguistic Auditory Milestone Scale (CAT/CLAMS, Capute), examining speech and language, fine motor/visual perceptual skills, and gross motor skills. Developmental delay and mental retardation was evident in all patients. In gross motor skills, 36% had a developmental quotient (DQ) of 50 or lower. Severe visual perceptual delay was seen in 43/55 (78%). Severe speech and language delay was found in 22% (N=12/55). IQ was determined by use of Weschler Intelligence Scale for Children (WISC), Stanford-Binet, or Weschler Adult Intelligence Scale (WAIS), depending on age. Eighty percent (N=44/55) of children had IQs between 40-70, in the mild to moderate range of mental retardation. Only one child had an IQ below 30. Vineland Adaptive Behavior Scales were administered on all subjects. Areas of communication, daily living, motor, and social skills were examined, and a composite score was given. Eighty-two percent had scores of less than 70, consistent with low adaptability. Communication and activities of daily living posed the greatest challenge. Children with SMS are affected with developmental delay and mental retardation with greatest deficits in language and visual-spatial skills. In addition, the majority of these children display poor adaptive behavior.

Program Nr: 110 from 2002 ASHG Annual Meeting

**Neuregulin 1 and susceptibility to schizophrenia.** *H. Stefansson[1], E. Sigurdsson[2], V. Steinthorsdottir[1], S. Bjornsdottir[1], T. Sigmundsson[2], J. Brynjolfsson[2], E. Gudmundsdottir[2], S. Gunnarsdottir[1], O. Ivarsson[2], V. Mutel[4], R. Harvey[5], A. Bjornsson[1], S. Sigfusson[2], M. Lemke[3], M. Frigge[1], M. Gurney[1], A. Kong[1], J. Gulcher[1], H. Petursson[2], K. Stefansson[1]*. 1) Decode Genetics, Reykjavik, Iceland; 2) Department of Psychiatry, National University Hospital, Reykjavk Iceland; 3) Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037; 4) F. Hoffmann-La Roche, Basel, Switzerland; 5) Faculties of Medicine and Life Sciences, University of New South Wales, Kensington, Sydney 2052, Australia.

The cause of schizophrenia is unknown but it has a significant genetic component. Pharmacologic studies, studies of gene expression in man, and studies of mouse mutants suggest involvement of glutamate and dopamine neurotransmitter systems. However, so far, strong association has not been found between schizophrenia and variants of the genes encoding components of these systems. Here we report the results of a genome-wide scan of schizophrenia families in Iceland that support previous work done in five populations showing that schizophrenia maps to chromosome 8p. Extensive fine-mapping of the 8p locus and haplotype association analysis, supplemented by a transmission disequilibrium test, identifies neuregulin 1 (NRG1) as a candidate gene for schizophrenia. NRG1 is expressed at central nervous system synapses and has a clear role in the expression and activation of neurotransmitter receptors, including glutamate receptors. We also demonstrate that knockouts for NRG1 or its receptor, ErbB4, show a behavioral phenotype that overlaps with mouse models for schizophrenia. Furthermore, NRG1 hypomorphs have fewer functional NMDA receptors than wild-type mice.

Program Nr: 111 from 2002 ASHG Annual Meeting

**THE FIRST GENOME-WIDE SCREEN IN ASPERGER SYNDROME: EVIDENCE FOR A LINKAGE ON CHROMOSOME 1q21-22 OVERLAPPING WITH A MAJOR SCHIZOPHRENIA LOCUS.** *I. Jarvela[1,2,3], T. Ylisaukko-oja[2,3], T. Nieminen-von Wendt[4], S. Sarenius[4], E. Kempas[2,3], M. Auranen[2,3], L. von Wendt[4], L. Peltonen[2,3,5].*
1) HUCH-Laboratory Diagnostics, Lab Molecular Genetics, Helsinki Univ Hosp, Helsinki, Finland; 2) Department of Molecular Medicine, National Public Health Institute, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Helsinki, Finland; 4) Unit of Child Neurology, Helsinki Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; 5) Department of Human Genetics, UCLA School of Medicine, Los Angeles, USA.

Asperger syndrome (AS) is a member of autism spectrum disorders (Pervasive Developmental Disorders; PDD). It is characterized by difficulties in social interaction and communication as well as the presence of circumscribed interests and activities. A two stage genome scan was performed in a total of 17 large Finnish AS-families (a total of 81 affected individuals) where the trait was inherited as an autosomal dominant trait. The diagnosis of the patients was based on ICD-10. All the patients were personally interviewed. In the first stage (12 families)nine loci (1q21-22, 3p14-24, 3q25-27, 4p15, 4q32, 6p25, 6q16, 13q31-33 and 18p11)were found to have Zmax > 1.5. In the second stage, these loci were analyzed with the complete family material and a denser set of microsatellite markers. The highest two-point lod score was observed on chromosome 1q21-22 (Zmax = 3.59)with several flanking markers giving a lod score >2.0. We also observed some evidence for a common shared haplotype extending 3 cM in three of the families on this region. Evidence for linkage on 1q21-22 has previously been reported in both schizophrenia (Brzustowicz et al. 2000; Gurling et al. 2001) and autism (Auranen et al. submitted). Interestingly, in five out of the 17 families the diagnosis of schizophrenia was found in three AS-patients and in two family members. These findings raise a question about a common genetic background between AS and schizophrenia. In fine mapping, Zmax of 2.52 on chromosome 3p14-24 was detected which is located near an autism locus (Buxbaum et al. 2001; Shao et al. 2002; Auranen et al. submitted).

Program Nr: 112 from 2002 ASHG Annual Meeting

**Autism: Physical features can predict which children respond to intensive ABA therapy.** *J.H. Miles, R. Yarnal, T.N. Takahashi, M. Stoelb, R. McCathren.* Dept Child Hlth, Med Gen Div, Univ Missouri Hosp, Columbia, MO.

Autism is a common neurodevelopmental disorder with long-term outcomes that vary from resolution of symptoms and adequate functioning to life-long impairment and dependence. Intensive, early behavioral training is touted as the most effective therapy with 47% of children responding favorably (Lovaas, 1987); however, the rest respond poorly or not at all. The current ability to predict outcome is very weak with previous studies reporting IQ (Stevens et al., 2000) & verbal skills (Venter et al., 1992) as the strongest predictors of academic achievement. In an effort to determine whether genetic heterogeneity within the autism diagnosis predetermines which children respond, we analyzed the outcomes of 20 children who completed one year of 22 hrs/wk 1:1 ABA therapy (sd=6.42, range 12-35.5). Mean age at the onset of treatment was 4.61 years (sd=1.97, range 2.16-10.16); all were Caucasian; 70% were male. Outcomes were based on 6-8 point progress scales in language, motor, social, and play skills. Subjects were divided into groups representing best outcome (n=6), good outcome (n=9), and poor outcome (n=5) based on natural gaps in progress determined by the difference of sums across all scales at treatment onset and at 1 year. Groups were compared against physical and functional variables including gender, age at therapy, type of autism onset, treatment intensity, seizures, normal or abnormal MRI, and SES classification. Groups differed significantly with the presence of dysmorphology ($p<.004$) and seizures ($p<.018$) more highly associated with a poor outcome. Of the group of 10 subjects notable for a complete lack of language skills at treatment onset, 5 made substantial progress and 5 made little or no progress: the absence of dysmorphology was found to be highly significant ($p<.001$) and to predict language skill acquisition with 100% accuracy. These outcomes support the proposition that autism is genetically heterogeneous and that physical markers of abnormal morphogenesis are a reliable predictor of outcome using intensive ABA therapy. This is exceptionally important for young, nonverbal children in whom IQ can not be accurately measured.

Program Nr: 113 from 2002 ASHG Annual Meeting

**Autism age-of onset quantitative trait loci (qtl).** *R.M. Cantor-Chiu[1], M. Alarcon[2], J.Y. Yuan[1], A.G.R.E. Consortium[3], D.H. Geschwind[2]*. 1) Human Genetics; 2) Neurology; UCLA School of Medicine, Los Angeles, CA; 3) Cure Autism Now.

Autism is a complex disorder identified by impaired language and social skills, restricted repetitive behaviors, and an early childhood onset. Monozygotic compared to dizygotic concordance rates indicate multiple interacting genes are likely. To reduce complexity, we are screening for 'potentially' genetic traits that may be more appropriate for gene mapping studies than the diagnosis of autism. Sibling correlations of quantitative items derived from the Autism Diagnostic Interview Revised (ADIR) were estimated in approximately 200 autistic sibpairs collected by the Autism Genetics Research Exchange (AGRE). Age when developmental abnormalities manifested, as assessed by the ADIR interviewer (A), ($r = .38$, $p < .0001$) and a score derived from items targeting repetitive ritualistic behaviors (R) ($r = .47$, $p<.0001$)were each significantly correlated in sibs, making them good candidates for qtl mapping. A and R are not correlated with each other ($r = -.03$) or with age at first word ($r = -.08$ and $r = -.01$, respectively), which is linked to the distal 7q region in this sample. A and R were analyzed using the nonparametric command of genehunter and a z score greater than 2.5 as the criterion for follow-up studies. For A, regions on chromosomes 1q ($z=2.60$, $p<.0047$), 10p ($z=3.05$, $p<.0012$) and 13p ($z=2.74$, $p<.0031$) were identified, and a broad peak with a weaker signal ($z=2.22$, $p=.013$) was seen on 7q in a region linked to autism in studies of independent samples and 'age at first word' in this sample. Although possibly less reliable, parental assessment of age when developmental abnormalities manifested was also analyzed. 3p ($z=3.25$, $p<.00058$) and 7q ($z=3.2$, $p<.00069$) were implicated. The strongest signals for R on 1p ($z=2.1$, $p<.018$) and 9q ($z=2.3$, $p<.011$) were lower than the follow up criterion. These findings may be interpreted as preliminary, however they highlight traits and chromosomal regions for fine mapping and replication in independent samples. 7q continues to be identified as harboring genes contributing to autism phenotypes. This approach illustrates the power of dissecting complex disorders into more specific familial phenotypes for gene mapping.

Program Nr: 114 from 2002 ASHG Annual Meeting

**An autism patient with a t(5;7)(q14;q31)translocation: localization, identification and mutation-screening of candidate genes at the breakpoints.** *J.B. Vincent[1,2], D. Kolozsvari[1], J. Cheung[1], M. Haddad[1], W. Roberts[3], S.W. Scherer[1]*. 1) Dept. of Genetics and Genomic Biology, Hospital for Sick Children, Toronto, ON, Canada; 2) Dept. of Psychiatry, University Toronto, ON, Canada; 3) Child Development Centre, Hospital for Sick Children, Toronto, ON, Canada.

We have identified a male autism patient with a balanced de novo translocation between chromosomes 5q14 and 7q31. The region on chromosome 7 has been implicated by a number of genome-wide scans, and is believed to harbour at least one susceptibility gene for autism. We have mapped the translocation breakpoints using fluorescence in situ hybridization (FISH), and identified a number of nearby candidate genes, which we have screened for mutations using denaturing high performance liquid chromatography in 96 unrelated autism probands. These genes include KCND2, TSA806 and LSm8 on 7q31.2, just distal to the CFTR locus. RT-PCR analysis for informative SNPs was performed for a number of these genes for this patient, in order to demonstrate monoallelic expression, showing that expression of the derivative copy of these genes may be disrupted in this patient. For KCND2, a gene encoding a brain-specific potassium voltage-gated channel, several amino acid sequence variants were identified, including one variant present in both affected and one unaffected sibling in one family, and another present in both affected siblings of a second family. Comparative genomic analysis across the translocation breakpoint region on 7q31 has revealed the presence of regions conserved between mouse and humans. Analysis of these conserved regions assisted the identification, by RT-PCR and sequence analysis, of a rare transcript situated close to the breakpoint site. We also identified a novel gene from this region, that spans the breakpoint on 7q31.2. This gene contains at least 8 exons, spans over 280 Kb, and shows a high level of alternative splicing. Further characterization of these new transcripts is now underway.

Program Nr: 115 from 2002 ASHG Annual Meeting

**Molecular Genetic Testing for Rett Syndrome.** *B.B. Roa[1], K. Bowles[1], I.M. Buyse[1], P. Fang[1], P. Arn[2], C.M. Bowe[3], R.A. Kern[4], H.Y. Zoghbi[1].* 1) Dept Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Nemours Children's Clinic, Jacksonville, FL; 3) Univ of California Davis, Sacramento, CA; 4) Ohio State Univ College of Medicine, Columbus, OH.

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that affects ~1/10,000-1/15,000 females. Patients appear to develop normally until 6-18 months, followed by regression of motor and language development. The Rett syndrome gene on Xq28 was identified as *MECP2*, which encodes the transcriptional repressor methyl-CpG-binding protein 2. *MECP2* mutations were found in ~80% of classic Rett patients, as well as females and males with a spectrum of neurodevelopmental phenotypes. Our DNA diagnostic laboratory tested >1700 patients using a strategy combining denaturing high-pressure liquid chromatography (DHPLC) and DNA sequencing. Over 99% concordance was observed between these two methods. To enhance testing efficiency, an initial screening panel for common *MECP2* mutations is being developed on a MALDI-TOF mass spectrometry platform in combination with DNA sequence analysis of negative patients. Various novel and recurrent *MECP2* mutations were identified in ~30% of patients we have tested. Interesting and unusual cases include a 10-year old female with atypical Rett features in whom a low-level nonsense mutation was identified (1093G>T, E365X; mutant to wildtype allele ratio ~18:82). This mosaic mutation was identified by DHPLC and confirmed by sequence analysis of fraction-collected DNA templates; similar results were obtained from patient lymphocytes, buccal cells, and fibroblasts. A second case involves a 13 y.o. male with a Rett syndrome presentation in whom a common missense mutation (473 C>T, T158M) was identified in the mosaic state. A third case involves a 2 y.o. female Rett patient in whom two different nonsense mutations were identified, 502C>T (R168X) and 880C>T (R294X). Sequencing of both parents were negative, indicating a de novo origin. Our collective data highlight the spectrum of *MECP2* genotypes and phenotypes, and support clinical testing in patients with Rett syndrome as well as overlapping phenotypes such as Angelman syndrome, autism, and uncharacterized mental retardation.

Program Nr: 116 from 2002 ASHG Annual Meeting

**Disruption of *ATRX* in mouse by conditional knockout.** *R.J. Gibbons[1], D. Garrick[1], J. Sharpe[1], W.G. Wood[1], D.J. Picketts[2], A.J.H. Smith[3], L. Dobie[3], D.R. Higgs[1].* 1) MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, Oxford University, Oxford, UK; 2) Ottawa Health Research Institute, Ottawa, Ontario, Canada; 3) Centre for Genome Research, University of Edinburgh, Edinburgh, UK.

Mutations in *ATRX*, cause in human males, a severe X-linked form of mental retardation associated with facial dysmorphism, genital abnormalities and alpha thalassemia. Female heterozygotes are, in contrast, phenotypically normal and this is associated with highly skewed X inactivation with the mutant allele on the inactive X.

The presence of alpha thalassemia is due to down regulation of alpha globin gene expression and it seems likely that ATRX plays a role in the regulation of gene expression. In addition, mutations are associated with perturbation of DNA methylation in specific heterochromatic regions (rDNA, the DYZ2 repeat, the telomeric sequence TelBam3.4). The ATRX protein, which is predominantly associated with heterochromatin, belongs to the SNF2 family of ATPases which appear to function via their capacity to remodel chromatin.

In order to generate an *ATRX* knockout in mouse, the Cre/lox system was used. The floxed allele was introduced into male ES cells by homologous recombination. Mice in which the floxed allele was transmitted in the germline were crossed with mice homozygous for Cre to generate offspring with *ATRX* disrupted . Interestingly, male offspring null for *ATRX* die in early embryonic development (7.5-8.5 dpc). Females heterozygous for the null allele are small, dysmorphic and exhibit abnormal behaviour. Recent evidence now suggests that contrary to our previous assumption, none of the human mutations are true nulls.

As with mutations in human *ATRX*, in mouse embryonic stem cells knocked out for *ATRX* by transient tranfection with a Cre expressing construct, methylation is perturbed. Like the SWI/SNF proteins DDM1 and lsh, ATRX would appear to provide an important link between the processes of chromatin remodelling and DNA methylation.

Program Nr: 117 from 2002 ASHG Annual Meeting

**Etiology of mental retardation in 281 children referred to a tertiary care center: A prospective study.** *C.D. van Karnebeek, F.Y. Scheper, N.G. Abeling, M. Alders, P.G. Barth, J.M. Hoovers, C. Koevoets, R.J. Wanders, R.C. Hennekam.* Pediatrics/Clinical Genetics, Academic Medical Center, Amsterdam, Amsterdam.

Aim: The objective of this etiologic study was to assess diagnostic possibilities in a consecutive cohort of children with unexplained mental retardation(MR) referred to a tertiary care center. Methods:In a prospective study (1998-2000)all patients with unexplained MR (IQ≤ 85;age≤ 17 yrs)were examined. Standard assessment included complete clinical history; a three-generation pedigree;physical examination;behavioural assessment.If clinical work-up findings suggested a particular diagnosis,appropriate confirmatory investigations were performed.If no diagnosis was evident,cytogenetic studies and metabolic investigations (urinary analysis; glycosylation+cholesterol screening)were performed.If normal, subtelomeric FISH analysis was performed.If normal,further additional investigations were performed. Results:281 children(162m)were included (mean age: 7.4yrs),with borderline(16.0%), mild(39.2%),moderate(31.3%), severe(10.3%),or profound(3.2%)MR. In 150 patients(54%), a diagnosis was established. Diagnostic categories were: teratogenic(5.9%); perinatal(0.7%), cytogenetic(20.4%); molecular(4.5%); metabolic(4.5%); clinical diagnoses with known etiology(7.8%); clinical diagnoses with unknown etiology but monogenic inheritance (38.3%); clinical diagnoses with non-monogenic or unknown inheritance(17.9%). One-third of diagnoses was established based on findings of clinical history(CH)+ physical exam(PE) only; for another 1/3 CH+PE provided essential clues to allow appropriate additional investigations; in the other 1/3 diagnoses were established by additional investigations only. The likelihood to reach a diagnosis did not depend on the severity of MR. The likelihood of detecting a chromosome anomaly was higher in patients with more(and more widespread) minor anomalies.A high number of malformations could also be found in children with metabolic disorders. Conclusions:In an outpatient teriary clinic, a diagnosis can be established in 1:2 MR patients referred for diagnostic evaluation.Diagnostic procedures in patients with unexplained MR are complex requiring a multidisciplinary approach.

Program Nr: 118 from 2002 ASHG Annual Meeting

***FACL4* is responsible for X-linked mental retardation in several families: proposal for a rapid screening of mentally retarded males.** *A. Renieri[1], I. Longo[1], C. Pescucci[1], F. Ariani[1], F. Mari[1], M. Bruttini[1], I. Meloni[1], E. XLMR Consortium[2]*. 1) Dept Molec Biol, Medical Gen, Univ Siena, Siena, Italy; 2) European XLMR Consortium.

Using a deletion map strategy on the contiguous gene deletion syndrome ATS-MR in Xq22.3, we demonstrated that *FACL4* which encodes fatty acid CoA ligase type 4, was a candidate gene for mental retardation (J Med Genet, 2002). Mutation analysis performed on affected males of family MRX63 demonstrated a missense mutation (p.R570S) significantly reducing enzymatic activity, and indicated that *FACL4* was indeed a gene responsible for nonspecific mental retardation (MRX) (Nat Genet, 2002). After the first screening, we have extended analysis of *FACL4* gene to 8 families affected by syndromic (6 families) and nonspecific (2 families) mental retardation, mapped in a large interval encompassing Xq22.3. We have found a novel missense mutation in another MRX family: MRX68. The identified mutation (c.1001C>T) in the brain isoform) co-segregates with the disease and changes a highly conserved proline into a leucine (p.P375L) in the first luciferase domain, which markedly reduces the enzymatic activity. Presently, *FACL4* is mutated in 2 MRX families, MRX63 and MRX68, and in another small family identified by screening 107 unrelated males with XLMR. Immunohistochemical experiments on adult human brain demonstrated that *FACL4* gene is highly expressed in brain, where it presents a brain-specific isoform, and localizes in cerebellar and hippocampal neurons at locations thought to be involved in memory formation. Very interestingly, all heterozygous females carrying either a point mutation in *FACL4* gene (8 females from 3 families) or a genomic deletion encompassing *FACL4* gene (3 females from 2 ATS-MR families) showed a completely skewed X-inactivation in blood leucocytes, suggesting that the gene influences survival advantage. Since mutations in *FACL4* are relatively frequent, we have developed a rapid enzymatic assay on peripheral blood that we propose as a sensitive, robust and efficient diagnostic tool for screening mentally retarded males. This work was supported by Telethon grant E.1145 to AR.

Program Nr: 119 from 2002 ASHG Annual Meeting

**Target genomic regions of MeCP2 protein associated with Rett syndrome.** *T. Kubota, T. Nomura, M. Matsumura, Y. Goto.* Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, NCNP, Tokyo.

Rett syndrome is an X-linked dominant disease caused by mutation of the MeCP2 gene. MeCP2 protein binds to methylated-CpG regions at the promoters of genes, and suppresses their expressions in vitro. Therefore, it is hypothesized that inappropriate upregulation of genes by failure of binding of the mutant MeCP2 to the methylated-CpG regions may cause neurological symptoms of this disease (e.g. autism, epilepsy). However, such CpG regions have not been identified. Therefore, to know the MeCP2-target genomic regions, we first performed immunofluorescence analysis with an anti-MeCP2 antibody on metaphase chromosomes of normal lymphoblasts. The analysis showed that MeCP2 protein was dispersed throughout the chromosome arms. This staining pattern was similar to that in rats, but was different from that in mice, which is specially localized in heterochromatin regions. Then, we performed a chromatin immnoprecipitation (ChIP) assay with the same anti-MeCP2 antibody using normal lymphoblasts. Sequence analysis of cloned MeCP2-binding genomic DNA fragments showed that 53 of the 93 fragments (57%) fulfilled the criteria of CpG island (over 50% of G+C content and high CpG frequency). Of these, two fragments were hit by BLAST search (12q13 and 14q32, respectively) and one of them was matched with a CpG island of a gene. This CpG island was confirmed in other ChIP products of normal lymphoblasts by PCR. These results indicate that MeCP2 protein can target many CpG regions in human genome, and the MeCP2-target sequence database may provide useful genomic-based information to identify the upregulated genes associated with Rett syndrome, in combination with the expression-based study using a DNA chip.

Program Nr: 120 from 2002 ASHG Annual Meeting

**A mouse model of Rett syndrome: evidence supporting an epigenetic mechanism of pathogenesis.** *J.I. Young[1], M.D. Shahbazian[1], P.M. Moretti[1], L.A. Yuva-Paylor[1], C.M. Spencer[1], J.L. Noebels[3], D.L. Armstrong[2], R. Paylor[1,5], H.Y. Zoghbi[1,4,6]*. 1) Department of Molecular and Human Genetics; 2) Pathology; 3) Neurology; 4) Pediatrics; 5) Neuroscience; 6) Howard Hughes Medical Institute. Baylor College of Medicine, Houston, TX.

Rett syndrome (RTT) is characterized by a normal period of development followed by loss of acquired speech and purposeful hand use, ataxia, apraxia, hand stereotypies, seizures, and autistic features. Mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2), a transcriptional repressor, account for 85% of classic RTT as well as other phenotypes (mental retardation, psychosis, infantile encephalopathy), depending on patterns of X chromosome inactivation (XCI) in females or the type of mutations in males. To generate a mouse model of RTT we targeted a truncating mutation (deleting the last 178 amino acids) to mimic mutations common in classic RTT. Mutant male mice recapitulate all major aspects of the disease. *MeCP2*$^{308/y}$mice appear normal until 6 weeks of age, when they display tremors and stereotypic forelimb motions resembling those of RTT patients. They also exhibit impaired coordination, hypoactivity, increased anxiety, and altered social interactions. At later ages, the mice develop kyphosis and are prone to myoclonic seizures. Females developed some of the same abnormalities at older ages; XCI patterns in the mutant females favored the wild-type allele. To determine if MeCP2s interactions with HDAC-containing complexes play a role in RTT pathogenesis, we evaluated patterns of histone acetylation in *MeCP2*$^{308/y}$ mice. Truncated MeCP2 localizes normally to heterochromatic domains, but its ability to modulate chromatin architecture is impaired: histone H3 is hyperacetylated in a tissue-specific manner that parallels MeCP2 abundance, with 2- to 3-fold increases in acetylated H3 in the cortex. These data show that partial loss of function of MeCP2 produces classic RTT in male mice and that such disease-causing mutations are likely to perturb chromatin architecture. These mice will allow us to explore epigenetic alterations caused by MeCP2 dysfunction and their relation to specific aspects of the RTT phenotype.

Program Nr: 121 from 2002 ASHG Annual Meeting

**The ITSN1 Knockout mouse generates cortical malformations as seen in deletion for human chromosome 21.** *X-N. Chen[1], F. Zhang[1], H.B. Sarnat[2], L. Flores-Sarnat[2], R. Jacobs[3], J.R. Korenberg[1].* 1) Med Genet, Cedars-Sinai Med Ctr, UCLA, Los Angeles, CA; 2) Peds Neurology, Cedars-Sinai Med Ctr, UCLA, Los Angeles, CA; 3) Biological Imaging Center, Beckman Institute, Caltech, Pasadena, CA.

Individuals with Down syndrome and aneuploidy for chromosome 21 have malformations of the neocortex, hippocampus and cerebellar cortex. Using a molecular analysis of 16 individuals with partial deletion of chromosome 21, we have identified a region of 21q22.1 that is associated with cortical dysplasia. This region contains the gene for intersectin 1 (ITSN1) involved in clathrin mediated endocytosis. In order to evaluate the contribution of ITSN1 to brain morphogenesis, we generated a knockout mouse and validated the genoptype by using PCR, genomic sequencing, and Southern blotting. The phenotype was characterized by using: 1) tissue hematoxylin and eosin staining (HE); 2) immunohistochemistry (IHC) with antibodies against ITSN1; and 3) mouse brain magnetic resonance imaging (MRI) (100m resolution). The preliminary results were impressive. Neuroanatomic evaluation by HE staining of coronal and sagittal sections of the brain in homozygotes, heterozygotes, and wild-type controls revealed the malformation of hippocampus, thinning and reduced lamination of the neocortex, and cerebellar hypoplasia in the homozygote, with milder malformations seen in the heterozygote. Greatly decreased ITSN1 staining was seen by IHC in the brain of the homozygote. To more clearly evaluate the nature of the complex cerebral malformations, we employed mouse MRI, which not only confirmed the malformation of hippocampus, but also illustrated significant abnormalities of the midline, including absence of corpus callosum. These first studies of the mouse support the important role for ITSN1 in brain development suggested by the human studies. What is unexpected is that the brain of the homozygous knockout functions in a manner compatible with life. These results illustrate the value of MRI for mouse models of human disease. The ITSN1 knockout model will provide insight into alternative neural pathways involving clathrin mediated endocytosis.

Program Nr: 122 from 2002 ASHG Annual Meeting

**Evolutionary changes in Microcephalin, a protein implicated in determining human brain size.** *A.P. Jackson[1,2], S.M. Bell[1], M. Houseman[1], I.M. Carr[1], H. Jafri[3], Y. Rashid[3], G. Elgar[4], A.F. Markham[1], C.G. Woods[1,2].* 1) Molecular Medicine Unit, CSB, University of Leeds, St. James's University Hospital, Leeds, LS9 7TF UK; 2) Department of Clinical Genetics, St. James's University Hospital, Leeds, LS9 7TF, UK; 3) Genetec Lab, 146/1 Shadman Jail Road, Lahore, Pakistan; 4) Fugu Genomics Project, Research Division, UK MRC HGMP Resource Centre, Hinxton, Cambridge, UK.

Primary microcephaly (OMIM 251200) is an autosomal recessive neurodevelopmental condition in which there is a global reduction in cerebral cortex volume to a size comparable with that of early hominids. This marked reduction in human brain size (e.g. 430g compared with 1450g in the normal adult) raises the question as to whether genes mutated in microcephaly may provide insights into the mechanisms by which brain size has dramatically increased during evolution.

We have identified a gene encoding a BRCA1 C-terminal domain-containing protein, mutated in MCPH1 families sharing an ancestral 8p23 haplotype. This gene, *microcephalin,* is expressed in the developing cerebral cortex of the fetal brain. Sequence homologies suggest a role in DNA repair or cell cycle regulation. The *microcephalin* gene has been present throughout vertebrate evolution, and we have characterised fish, rodent and primate orthologs. All contain the *angiopoeitin-2* gene on the reverse strand in intron 12 of *microcephalin*. Protein sequence homology is strikingly low, with 57% sequence identity between mouse and human orthologs (c.f. mean human-mouse protein sequence identity, 85%; angiopoeitin-2, 88%). This suggests the potential for significant functional changes during recent evolution.

Further studies of microcephalin in different species should provide insights into the regulation of cortical neural cell number, potentially aid understanding of neural progenitor/stem cell regulation and shed light on the evolution of the cerebral cortex.

Program Nr: 123 from 2002 ASHG Annual Meeting

**Identification of the MCPH5 gene suggests an evolutionary mechanism in the control of brain size.** *J. Bond[1], E. Roberts[1], G.H. Mochida[2,3], J.M. Askham[1], C.A. Walsh[2], C.G. Woods[1,4].* 1) Molecular Medicine Unit, St. James's University Hospital, Leeds, W. Yorkshire, U.K; 2) Division of Neurogenetics, Harvard Medical School, Boston, MA, USA; 3) Department of Neurology, Massachusetts General Hospital, Boston, MA, USA; 4) Department of Clinical Genetics, St Jamess University Hospital, Beckett Street, Leeds LS9 7TF, UK.

One of the most notable trends in mammalian evolution is the massive increase in size of the cerebral cortex, especially in higher primates. Any human disorder affecting cerebral cortex size offers potential insights into both its development and evolution. We are studying the human disease autosomal recessive primary microcephaly (MCPH), which is characterized by a small, but otherwise grossly normal, cerebral cortex and mild to moderate mental retardation. MCPH exhibits genetic heterogeneity, with at least five recessive loci known. Of these MCPH5 (1q31) is the most prevalent, accounting for 24 of 56 consanguineous Northern Pakistani families and is also the only MCPH locus known to affect multiple ethnic groups. Using autozygosity mapping, common haplotype identification and a positional cloning strategy we have identified the human MCPH5 gene. Current work involves phenotype-genotype comparisons, determination of the mutation spectrum of the MCPH5 gene, expression analysis and investigation of gene conservation in organisms with a central nervous system. Interspecies comparisons of the predicted MCPH5 proteins show remarkable overall conservation, but a consistent increase in protein size in species with larger brains.

Program Nr: 124 from 2002 ASHG Annual Meeting

**Amish microcephaly is caused by mutation in the deoxynucleotide carrier *DNC*.** *M.J. Rosenberg[1], R. Agarwala[1], G. Bouffard[1], J. Davis[1], G. Fiermonte[2], M.S. Hilliard[3], T. Koch[4], L.M. Kalikin[5], I. Makalowska[1], D.H. Morton[6], E.M. Petty[5], J.L. Weber[7], F. Palmieri[2], R.I. Kelley[8], A.A. Schäffer[1], L.G. Biesecker[1]*. 1) National Institutes of Health, Bethesda, MD; 2) Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari, Bari, Italy; 3) Logicon R.O.W. Sciences Corporation, Rockville, MD; 4) Konrad-Zuse-Zentrum für Informationstechnik, Berlin Germany; 5) University of Michigan Medical Center, Department of Internal Medicine, Ann Arbor, MI; 6) The Clinic for Special Children, Strasburg, PA; 7) Marshfield Medical Research Foundation, Marshfield, WI; 8) Kennedy-Krieger Institute and Department of Pediatrics, Johns Hopkins University, Baltimore, MD.

Amish microcephaly (MCPHA) comprises severe microcephaly, a congenital malformation of the brain, increased urinary a-ketoglutarate, and premature death. The disorder is inherited in an autosomal recessive pattern and has been observed only in Old Order Amish families who have ancestors in Lancaster County, Pennsylvania. At least 61 affected infants have been born in the last 40 years in 23 nuclear families. Using a genealogy database and automated pedigree software, we constructed a pedigree connecting all affected nuclear families to an ancestral couple. Using positional cloning, we identified a gene with a single nucleotide substitution that segregates with the disease in affected individuals and alters an amino acid highly conserved in similar proteins. Analysis of 252 control chromosomes showed no evidence of this substitution. This gene, *DNC*, encodes a deoxynucleotide carrier that is located in the inner mitochondrial membrane. Functional analysis of the mutant protein revealed that normal transport activity was lost. We hypothesize that insufficient transport of dNTPs into mitochondria in the developing central nervous system interferes with mitochondrial DNA synthesis, causing abnormal brain growth and development. Taken together, these data show that mitochondrial deoxynucleotide transport is critical for brain development.

Program Nr: 125 from 2002 ASHG Annual Meeting

**Identification of genes responsible for X linked mental retardation (XLMR) using a human X chromosome specific cDNA microarray.** *T. Wang[1], L. Zhang[1], C. Obie[2], S. Mousses[3], J. Trent[3], D. Valle[1,2].* 1) Institute of Genetic Medicine and Department of Pediatrics, Johns Hopkins University, Baltimore, MD; 2) Howard Hughes Medical Institute; 3) Cancer Genetics Branch, NHGRI, Bethesda, MD.

Mental retardation is the most common cause of severe handicaps in children and young adults, affecting 2-3% of the general population. X-linked mental retardation (XLMR) occurs in 1 in 600 males and is genetically heterogeneous. Among the 150-200 responsible loci on the X chromosome, less than 40 genes have been cloned. Delineation of the molecular basis of XLMR will aid in clinical management and contribute to our understanding of human cognitive function. Current methods of identifying XLMR genes by linkage are limited by the rarity of individual XLMR phenotype and genetic heterogeneity. We developed a strategy of using a human X chromosome-specific cDNA microarray to identify candidate genes responsible for XLMR by detecting mutations that result in a change in the abundance of mRNA. This approach has the advantage of quantitative analysis of gene expression in multiple samples and does not require large pedigrees. We produced a human X chromosome-specific cDNA microarray that includes 70 - 80% of genes on the X chromosome. A proof of principle study was done using a lymphoblast cell line from a male with chronic granulomatous disease by gene deletion. In a preliminary survey of lymphoblast cell lines from 32 XLMR probands, we identified more than 30 candidate genes with greater than 4 fold mRNA reduction. mRNA reduction was confirmed in XLMR probands in at least two genes (XC-03, XC-11) by Northern blot and quantitative PCR analysis. A mutation (-113 C > A) was identified in the promoter of XC-03, which alters the core-binding site for transcription factors, Elk-1 and Ets-1, and is associated with mRNA reduction in the lymphoblast cell lines. We are now conducting genetic and functional characterization of this mutation and other candidate genes. Our study shows that cDNA microarray is a valuable approach to study primary genetic defects on the human X chromosome. (Supported in part by a research grant from the American Academy of Pediatrics).

Program Nr: 126 from 2002 ASHG Annual Meeting

**Novel Manifestations of Bardet-Biedl Syndrome (BBS) in a Population Based Study.** *J. Green[1], S. Moore[1], E. Dicks[1], B. Fernandez[2], Y. Fan[3], A. Bhogal[3], W. Davidson[3], D. Macgregor[3], L. Penney[3], J. Dean[4], P. Parfrey[1]*. 1) Memorial University of Newfoundland, St John's, NF; 2) Newfoundland & Labrador Medical Genetics Program, St John's, NF, Canada; 3) Simon Fraser University, Vancouver, BC, Canada; 4) Medical Genetics, Grampian University Hospitals, Aberdeen, UK.

BBS (OMIM:209900) is associated with a wide variety of clinical manifestations, including retinal dystrophy, obesity, renal abnormalities, dystrophic extremities and hypogenitalism. The phenotype shows considerable inter- and intra-familial variation. To describe the dysmorphology and range of clinical features in BBS, the medical histories of a cohort of 46 patients with BBS from 26 families in Newfoundland were reviewed. In addition, 25/46 patients were examined in 2001. Six patients from 5 families were linked to the BBS1 locus on 11q13, one to 16q21 (BBS2), 5 patients from 1 pedigree linked to 3p12 (BBS3), and 5 patients from another family linked to 2q31 (BBS5). In a further 12 patients from 6 families, a mutation was identified in the *MKKS* gene associated with BBS6. The genotype was unknown in the other 17/46 (37%) patients from 12 families (2 patients from 1 family were excluded from all loci). Characteristic cranio-facial dysmorphic features included bitemporal narrowing, broad zygomata, short, narrow palpebral fissures, high palate, small, downturned mouth, long ears, and brachycephaly and/or macrocephaly. Neurologic abnormalities included impaired co-ordination (20/21, 95%), a wide-based, unsteady gait (20/23, 87%), paucity of facial muscle movement (15/20, 75%), limited extraocular eye movements (14/20, 70%) and an inability to smile voluntarily (4/19, 21%). Psychiatric illness occurred in 14/41 (34%). 16/45 (35%) had a history of cholecystectomy, and in 13/27 (48%) liver function tests were abnormal. Other medical problems were: asthma (14/43, 33%), hyperhydrosis (10/30, 33%), recurrent otitis media (12/42, 29%), epilepsy (13/45, 13%), thyroid disease (6/46, 13%), and idiopathic edema (6/45, 13%). There was no correlation with genotype. This extension of the phenotype suggests that the BBS genes are involved in the embryogenesis of most organ systems.

Program Nr: 127 from 2002 ASHG Annual Meeting

**Differential gene expression in sporadic macronodular adrenal hyperplasia compared to normal adrenals by microarrays : Dissecting the genetics of adrenocortical tumorigenesis.** *I. Bourdeau[1], A. Lacroix[2], L.S. Kirschner[1], N.G. Costouros[3], D. Lorang[3], C.A. Stratakis[1]*. 1) UGEN, DEB,NICHD, NIH, Bethesda, MD; 2) Hotel-Dieu CHUM, Montreal, Canada; 3) Surgery Br,NCI, Bethesda, MD.

Adrenal Cushing's syndrome (CS) may be caused by unilateral adenomas, carcinomas or bilateral adrenal hyperplasia. (primary pigmented nodular adrenal disease (PPNAD) and massive macronodular adrenal hyperplasia (MMAD). In MMAD, cortisol secretion may be mediated by various non-ACTH circulating hormones as GIP leading to food-dependent CS. The etiology of adrenal aberrant expression of hormone receptors remain unclear. We identified new candidates genes that may be involved in the pathogenesis of MMAD using cDNA microarray. We extracted total (t)RNA from MMAD tissues; control adrenal tRNA was from normal adrenal glands. (62 caucasians). Fluorescent labeled cDNA probes were hybridized to glass slides printed with 10,368 features containing clones involved in oncogenesis. The experiment was performed with reciprocal labeling of fluochromes. Tumor samples were obtained from 9 patients with CS due to MMAD. Six patients were screened in vivo for the presence of aberrant receptor expression; 3 GIP, 1 adrenergic,1 vasopressin and 1 LH/hCG. Microarray data was filtered. Genes had to be up- or down-regulated at a cutoff ratio of 3.0. Hierarchical clustering revealed two clusters of patients: GIP-dependent and non-GIP dependent CS. 99 and 21 genes were found to be up- and down-regulated, respectively. Among the up-regulated genes, hypoxia-inducible factor 1 (HIF1A), regulator of G-protein signalling 1(RGS1) and genes coding for proteins involved in cytoskeleton and gap-junction formation. A subgroup of neuroendocrine-related genes were differentially expressed between GIP-dependent and GIP-independent MMAD. Then MMAD tissues were stained for 2 neuroendocrine markers; synaptophysin (SYN) and chromogranin A. Immunostaining for chromogranin A was negative; however, SYN was positive. We conclude that we identified new potential candidates genes involved in MMAD pathophysiology. Neuroendocrine differentiation may underlie the phenomenon of ectopic expression of various hormone receptors in MMAD tissues.

Program Nr: 128 from 2002 ASHG Annual Meeting

**A conditional mouse knockout model of Multiple Endocrine Neoplasia, type 1.** *J.S. Crabtree[1], S.K. Libutti[2], D. Lorang[2], J.M. Ward[2], L. Garrett-Beal[1], P.C. Scacheri[1], D. Hanahan[3], H. Edlund[4], M.A. Magnuson[5], S.C. Chandrasekharappa[1], S.J. Marx[6], A.M. Spiegel[6], F.S. Collins[1]*. 1) NHGRI/NIH, Bethesda, MD; 2) NCI/NIH, Bethesda, MD; 3) UCSF, San Francisco,CA; 4) Univ. of Umea, Umea, Sweden; 5) Vanderbilt Univ., Nashville, TN; 6) NIDDK/NIH, Bethesda, MD.

Multiple endocrine neoplasia, type 1 (MEN1) is an autosomal dominant cancer syndrome characterized primarily by multiple tumors in the parathyroid, endocrine pancreas and anterior pituitary. Affected families are found to have germline loss of function mutations in the *MEN1* gene, and tumors are found to have lost the wildtype allele. A conditional knockout model was generated in which loxP sites were placed into introns 2 & 8 of the *Men1* gene. These floxed mice were crossed with transgenic mice expressing cre driven by the parathyroid hormone promoter (PTH-Cre) and three different lines of mice with cre expression driven by the rat insulin promoter (RIP-Cre). This results in tissue specific deletion of exons 3-8. By 28 weeks, PTH-Cre mice homozygous for the floxed *Men1* allele develop hypercalcemia. PTH levels are variably elevated, parathyroid hyperplasia occurs and PCR analysis shows deletion of exons 3-8 in some, but not all, parathyroid tissue. RIP-Cre mice with floxed *Men1* alleles develop a range of pancreatic lesions from islet hyperplasia to atypical islet hyperplasia to frank adenoma as early as 28 weeks, with homozygous mice showing more rapid disease progression than heterozygote littermates. Consistent with this, homozygotes demonstrate perturbations in blood chemistry at earlier ages than littermate heterozygotes. Fasting insulin levels in homozygotes increase with tumor onset and are often accompanied by a dramatic decrease in fasting blood glucose levels. Tumors developing in these mice lack menin, the *Men1* gene product, and express insulin, as evaluated by laser capture microdissection and immunohistochemistry. Use of this knockout leads us to propose that, in the pancreas, loss of one *Men1* allele results in islet hyperplasia, loss of both *Men1* alleles results in atypical islet hyperplasia, and additional, unknown somatic changes at other loci are required for frank tumor formation.

Program Nr: 129 from 2002 ASHG Annual Meeting

**Using RNAi and microarrays to investigate the function of menin.** *P.C Scacheri[1], N. Caplen[1], D. Shanmugarajah[1], J.S. Crabtree[1], S. Chandrasekharappa[1], S. Marx[2], A. Spiegel[2], F.S. Collins[1]*. 1) NIH, NHGRI, Bethesda, MD; 2) NIH, NIDDK, Bethesda, MD.

Multiple endocrine neoplasia, type I (MEN1), is a rare inherited cancer syndrome characterized by multiple tumors of the parathyroid glands, the pituitary gland, and the endocrine cells of the pancreatic islets and duodenum. The complete function of menin, the protein product of the *MEN1* gene, is unknown, but previous reports suggest that menin may interact with *JunD*, a transcriptional activator, and *smad3*, a member of the TGF-b signaling pathway. In an effort to determine the physiological role of menin in tumorigenesis, we are using siRNA duplexes (of 22 nucleotides) to suppress the expression of *MEN1* in HeLa cells. Fluorescent tagging of the *MEN1*-dsRNA allows FACS sorting of the transfected cells, allowing a nearly pure preparation of highly transfected cells to be analyzed for gene expression. HeLa cells transfected with *MEN1*-dsRNA show >90% reduction of menin protein at 72 hours following transfection compared to *CAT*-dsRNA transfected cells (negative control). Furthermore, compared to *CAT*-dsRNA transfected cells, *MEN1*-dsRNA transfected cells show a significantly decreased response to TGF-b, a growth inhibitory pathway hypothesized to require functional menin. Use of Affymetrix microarrays to compare the expression profiles of *MEN1*-dsRNA and *CAT*-dsRNA transfected cells reveals 38 genes that show greater than a two-fold difference in expression. Genes of interest include an insulin-induced gene (*INSIG1*), a TGF-b responsive gene (*TSC-22*), as well as several metallothionein genes that are known to be overexpressed in human tumors. These results provide insights into the early effects of loss of menin on global gene expression, which may ultimately reveal the role of menin in tumorigenesis.

Program Nr: 130 from 2002 ASHG Annual Meeting

**A Genome-wide Linkage Scan for Dietary Energy and Energy Nutrient Intake:The HERITAGE Family Study.**

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Diet intake is one of the risk factors for several chronic diseases such as obesity, cardiovascular disease, hypertension, and some forms of cancer. Besides an important effect of environmental factors, dietary energy and energy nutrient intake are partially determined by genetic factors. To identify potential candidate genes affecting total energy, carbohydrate, protein and fat intake we performed a genomic scan in 347 sibling pairs from 99 White families and 99 sibling pairs from 126 Black families. Dietary energy and energy nutrient intake, were assessed by Willetts Food Frequency Questionnaire. Single- and multipoint Haseman-Elston regression technique was used to test for linkage. Significant linkage ($p<0.00002$) according to Lander and Kruglyak was found for fat intake in Whites in chromosome 1p21.2 (multipoint) and chromosome 12q14.1 (single-point). Suggestive ($p<0.00007$) and promising ($p<0.0023$) linkages for energy, carbohydrate, fat, and protein intake were found in 20q13.13-13.2. Promising linkages were also found in chromosomes 7q31.3 in Whites and 12q23, 1q31.3, 1q32.1, 7q11.1 and 12q24.21 in Blacks. Several potential candidate genes are contained in and around the linkage regions in chromosome 1p21.2, 12q14.1, and 20q13.13-13.2 of Whites. These are the first data reported on the genomic scan of dietary energy and energy nutrient intake. Further linkage and disequilibrium studies are needed to better define regions and actual genes affecting diet intake.

Program Nr: 131 from 2002 ASHG Annual Meeting

**Scanning the Genome for Obesity Susceptibility Genes in Type 2 Diabetes Patients from West Africa.** *G. Chen[1], J. Zhou[1], A. Doumatey[1], A. Amoah[2], J. Acheampong[3], K. Agyenim-Boateng[3], B. Eghan Jr.[3], J. Oli[4], G. Okafor[4], E. Ofoegbu[4], B. Osotimehin[5], F. Abbiyesuku[5], T. Johnson[6], O. Fasimade[6], Y. Chen[1], H. Daniel[1], P. Furbert-Harris[1], G. Dunston[1], F. Collins[7], C. Rotimi[1] and Africa America Diabetes Mellitus (AADM) Study.* 1) National Human Genome Center, Howard University, washington, DC; 2) University of Ghana, Accra; 3) University of Science and Technology, Ghana; 4) University of Nigeria, Enugu; 5) UCH, Ibadan, Nigeria; 6) University of Lagos, Nigeria; 7) NHGRI, NIH, Bethesda.

Using the GENEHUNTER2 program, we conducted a genome search for susceptibility genes to multiple obesity traits (Body mass index -BMI, fat mass -FM, percent fat mass PFM) in a cohort of diabetes patients from five centers in West Africa (Ghana: Accra and Kumasi; Nigeria: Enugu, Ibadan and Lagos). A total of 341 families including 691 diabetes cases were studied. The strongest evidence of linkage was obtained for FM (LOD = 4.0, P = 0.000009) between D2S2739 and D2S441 followed by a LOD score of 3.3 (P = 0.000049) for PFM between markers D2S2739 and D2S441 on chromosome 2. Strong linkage signals were also obtained for BMI on chromosome 4 (LOD = 3.4; P= 0.000038) between markers D4S1647 and D4S2623; and for FM on chromosome 5 (LOD = 3.5; P = 0.00003) near marker D5S1725. All analyses were adjusted for gender, age and age. The Quantitative transmission/disequilibrium test (QTDT) demonstrated evidence of association between markers and obesity traits in these linkage regions. Interestingly, our linkage signals lie in regions that have been implicated as harboring obesity susceptibility genes in several previous studies conducted in multiple populations and ethnic groups. In conclusion, we found and replicated strong evidence of linkage and association with BMI on chromosome 4, with PFM on chromosome 5 and with FM on chromosomes 2 and 5 in our cohort of diabetes patients from West Africa one of the ancestral populations of African Americans.

Program Nr: 132 from 2002 ASHG Annual Meeting

**Loss-of-function mutations in the human *GLI2* gene cause holoprosencephaly and familial pan-hypopituitarism.**

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Defective Hedgehog function leads to holoprosencephaly (HPE) in humans and to cyclopia in mice. The secreted factor Sonic hedgehog (SHH) is a crucial signal for the patterning of the forebrain during vertebrate development and its normal function leads to the division of the primordial single eye field and the primitive telencephalon. The activity of the SHH pathway is mediated by transcription factors of the GLI family, which regulate the expression of target genes. In order to better understand the relative roles of *GLI* genes in the pathogenesis of HPE, we determined the gene architecture of human *GLI2* and analyzed a panel of 390 familial and sporadic HPE patients for evidence of sequence alterations. Here we report the identification of seven sequence variations in *GLI2* (not found in 200 control chromosomes). Functional studies in the frog embryo indicate that four mutations lead to loss of function. Two mutations are predicted to truncate the zinc-finger domain, as would the third mutation in the invariant splice donor consensus sequence. The fourth mutation segregating in a large pedigree associated with pan-hypopituitarism shows a frameshift predicted to produce a C-terminal deletion, which might be predicted to yield dominant negative function. This fourth human mutation disrupts GLI2 at a position similar to that of two zebrafish *Gli2* mutants (designated *you-too*) that are associated with a defective hypothalamus. Preliminary analysis, however, does not support dominant negative effects of this allele. Functional studies of sequence variations have proven essential, since three variations that appeared to be unique retained normal activity in frog *in vivo* assays. Our results provide evidence for an involvement of GLI2 in mediating SHH signaling during ventral brain development in humans and haploinsufficiency of GLI2 function resulting in HPE and pan-hypopituitarism.

Program Nr: 133 from 2002 ASHG Annual Meeting

**Roles of homeobox genes in pituitary development and disease revealed by allelic series in mice.** *S.A. Camper, I.O. Nasonkin, L.T. Raetzman, H. Suh, R.D. Ward, M.A. Charles, L.J. Cushman, P.J. Gage.* Dept. Human Genetics, 4301 MSRB III, Univ Michigan Medical School, Ann Arbor, MI.

*Prop1* and *Pitx2* are among the homeobox genes that are critical for normal pituitary development and function. Lesions in PROP1 affect only the pituitary and are the most common cause of multiple pituitary hormone deficiencies in humans. *PITX2* defects cause Rieger syndrome, which includes defects of the eyes, teeth, heart, umbilicus, and pituitary. Mouse models have been invaluable in the identification and functional analysis of these critical genes. A variety of alleles can be generated that vary in severity, allowing the dosage effect of genes to be ascertained, and alleles can be produced so that the genetic deficiency is induced at a particular time of development or in a specific tissue. We report comparison of a new loss of function allele of *Prop1* with two existing alleles. Complete loss of function causes alterations in the pituitary adrenal axis, respiratory distress and neonatal lethality. Partial loss of function is compatible with life but causes lack of GH, TSH, PRL and reduced gonadotropins. Gain of function causes transient hypogonadism, adult onset hypothyroidism and pituitary adenoma formation. A series of *Pitx2* alleles revealed distinct roles for PITX2 at different developmental times. It is required early for expansion of the pituitary primoridium and later for differentiation of the gonadotrope and *Pit1* lineages. We report the mechanism of hypocellularity in *Pitx2* null mice, and cell specification abnormalities in response to excess PITX2. This demonstrates the requirement for a precise level of PITX2 rather than achievement of a threshold level. Genetic analysis of these and other mouse models has revealed interactions between many of the critical genes and suggests a molecular basis for the complexities in human endocrine disease.

Program Nr: 134 from 2002 ASHG Annual Meeting

**Mutations in a novel PHD finger gene, *PHF6*, cause Börjeson-Forssman-Lehmann syndrome.** *K.M. Lower[1,2], G. Turner[3], B.J. Kerr[4], K.D. Mathews[5], M.A. Shaw[1], S. Schelley[6], H.E. Hoyme[6], S.M. White[7], M.B. Delatycki[7], A.K. Lampe[8], J. Clayton-Smith[9], H. Stewart[9], C.M.A. van Ravenswaay[9], B.B.A. de Vries[9], B. Cox[9], M. Grompe[9], S. Ross[9], P. Thomas[9], J.C. Mulley[1], J. Gécz[1,2]*. 1) Department of Cytogenetics & Molecular Genetics, Centre for Medical Genetics, Women's & Children's Hospital, North Adelaide, South Australia, Australia; 2) Department of Paediatrics, University of Adelaide, South Australia, Australia; 3) Hunter Genetics and University of Newcastle, New South Wales, Australia; 4) Centre for Neuroscience Research, Guy's, King's and St. Thomas' School of Biomedical Sciences, London, UK; 5) Department of Pediatrics and Neurology, University of Iowa Hospital and Clinics, Iowa, USA; 6) Division of Medical Genetics, Department of Pediatrics, H-315, Stanford University School of Medicine, CA, USA; 7) Genetic Health Services Victoria, Royal Children's Hospital, Melbourne, Australia; 8) Department of Clinical Genetics, Institute of Human Genetics, International Centre for Life, Newcastle upon Tyne, UK; 9) Other International Institutions.

Börjeson-Forssman-Lehmann syndrome (BFLS; MIM 301900) is a form of intellectual disability affecting predominantly boys. It is one of >100 similar disorders of the human brain mapped to the X chromosome. BFLS is characterised by moderate to severe mental retardation, hypogonadism, obesity, gynecomastia, short stature and characteristic craniofacial features. Previously, linkage analysis localized the gene to Xq26-q27. Using X-inactivation studies and deletion mapping, we reduced the interval containing the putative BFLS gene to ~9 Mb. Transcription mapping of this region identified at least 61 genes, 18 of which screened negative in BFLS patients. Among the rest, a novel, widely expressed PHD-like zinc finger gene, *PHF6*, had 8 different missense and truncation mutations in four familial and five sporadic cases of BFLS. GFP-tagged *PHF6* shows diffuse nuclear staining with prominent nucleolar accumulation in transiently transfected HeLa and Cos-7 cells. Such localisation, together with the presence of two PHD-like zinc fingers, is consistent with a role of the BFLS protein in regulation of transcription.

Program Nr: 135 from 2002 ASHG Annual Meeting

**Genotype-Phenotype relationships in Berardinelli-Seip congenital lipodystrophy.** *L. Van Maldergem[1], J. Magre[2], T. Gedde-Dahl Jr[3], E. Khallouf[4], E. Seemanova[5], M. Lathrop[6], J. Capeau[2], S. O'Rahilly[7] and the Berardinelli-Seip Study Group.* 1) Centre de Genetique Humaine, Institut de Pathologie et de Genetique Loverval; 2) INSERM U409, Facult de Medecine St Antoine, Paris, France; 3) Dept of Forensic Medicine Rikshospitalet, Oslo, Norway; 4) Dept of Endocrinology, Hotel Dieu de France, Beirut, Lebanon; 5) Dept of Medical Genetics, St Charles Hospital, Motol, Czech Republic; 6) Centre National de Genotypage, Evry, France; 7) Dept of Medicine and Clinical Chemistry, Addenbrooke Hospital, Cambridge, United-Kingdom.

Berardinelli-Seip Generalized lipodystrophy is a rare autosomal recessive human disorder with severe adverse metabolic consequences. A locus on chromosome 9 (BSCL1) has recently been identified, predominantly in African-American families, corresponding to AGTAP2 gene. Mutations in a previously undescribed gene of unknown function (BSCL2) on chromosome 11, termed seipin, have also been found to be responsible for this disorder in a number of European and Middle Eastern families. We have studied the genotype/phenotype relationships in 71 affected subjects from 44 apparently unrelated pedigrees. In all subjects, hepatic dysfunction, hyperlipidemia, diabetes mellitus and hypertrophic cardiomyopathy were significant contributors to morbidity with no clear differences between subjects with BSCL1, BSCL2 and those with evidence against linkage to either chromosomes 9 or 11 (designated BSCLX). BSCL1 is a less severe disorder than BSCL2 with a higher frequency of subjects with delayed onset of lipodystrophy. Notably, subjects with BSCL2 had a significantly higher prevalence of intellectual impairment (36/45) than those with BSCL1 (2/22) ($p<0.001$) or BSCLX (0/3). In summary, generalized lipodystrophy encompasses at least three loci. While the consequences for metabolic derangement, hepatic dysfunction and cardiac enlargement appear similar between these three groups, subjects with seipin mutations (BSCL2) appear to have a markedly higher prevalence of intellectual impairment and a higher incidence of premature death, findings which have major implications for genetic counseling.

Program Nr: 136 from 2002 ASHG Annual Meeting

**Concordant results of microarray and gene mapping studies in human autoimmune disease.** *T.M. Aune[1], J.S. Parker[2], K. Maas[1], N. Olsen[1], J.H. Moore[2].* 1) Medicine, Vanderbilt University, Nashville, TN; 2) Program in Human Genetics, Vanderbilt University, Nashville, TN.

Autoimmune diseases are thought to arise from complex interactions between environmental and genetic factors. Using cDNA microarrays, we have measured expression levels of > 4,000 genes in peripheral blood mononuclear cells of individuals with different autoimmune diseases. We find highly conserved changes in the expression of a select group of genes in all individuals with autoimmune disease independent of the type of autoimmune disease and its disease manifestations. Here, we show that locations of these differentially expressed genes in the genome are distributed in a significantly nonrandom fashion in specific clusters or chromosomal domains. These clusters significantly overlap with disease susceptibility loci that are identified by genetic linkage studies to be shared among multiple autoimmune diseases. This study demonstrates concordance between the physical location of genes that are differentially expressed in four common autoimmune diseases and loci that are linked to autoimmune disease susceptibility and are shared among multiple autoimmune diseases. These studies may provide a foundation for identifying specific genes that contribute to disease susceptibility. They may also help define genes that differ in expression levels and genes that exhibit differences in coding regions. To our knowledge, this is the first study showing concordance between gene expression and gene mapping results in a common complex multifactorial human disease.

Program Nr: 137 from 2002 ASHG Annual Meeting

**An inflammatory bowel disease genome scan stratified by Crohn's disease associated variants reveals susceptibility loci, genetic heterogeneity and epistasis.** *D.A. van Heel[1], B.M. Dechairo[2], G. Dawson[2], D.P.B. McGovern[1], K. Negoro[1], A.H. Carey[2], L.R. Cardon[1], I. MacKay[2], D.P. Jewell[1], N.J. Lench[2].* 1) Wellcome Trust Centre for Human Genetics and Gastroenterology Unit, University of Oxford, Oxford, UK; 2) Oxagen Ltd., Abingdon, UK.

**Background and Aims:** Genetic studies in inflammatory bowel disease have identified multiple susceptibility loci. The significance of these findings depends on verification in independent cohorts. Genetic variants associated with Crohns disease have now been identified on chromosomes 5 (IBD5 risk haplotype) and 16 (IBD1 locus, CARD15/NOD2 mutations). These variants now allow stratification of linkage analyses, which will improve the ability to identify other loci, and allow assessment of of potential complex interactions between genetic factors. **Methods:** We performed a genome-wide scan of 228 inflammatory bowel disease families. Multipoint linkage was assessed using MERLIN for inflammatory bowel disease (IBD, 288 affected relative pairs), Crohns disease (CD, 137 pairs) and ulcerative colitis (UC, 95 pairs) phenotypes. CD analyses were further stratified by common CARD15/NOD2 mutations and the IBD5 haplotype. **Results:** We observed linkage between UC and chromosome 5q (LOD 1.7), and confirmed loci on chromosomes 3q (CD, LOD 1.9) and 6p (IBD, LOD 2.0). Stratification by non-possession of common CARD15 mutations uncovered CD loci on chromosomes 17p (LOD 1.5), 19q (LOD 2.7) and Xp (LOD 1.8). The chromosome 19q locus, also demonstrated in a Canadian population, showed significant genetic heterogeneity with CARD15 (P=0.002) and epistasis with the IBD5 haplotype (P=0.02, LOD 2.3 in carriers). **Conclusions:** Our data, taken together with other studies in IBD, suggests susceptibility loci on chromosomes 3,6,17,19 and X. Stratification by associated variants demonstrates the complex genetic basis to IBD, with heterogeneity and epistasis between susceptibility loci.

Program Nr: 138 from 2002 ASHG Annual Meeting

**Functional analysis of genetic variations of CARD15 in Crohn's Disease predisposition.** *M. Chamaillard[1,2], D. Philpott[4], F. Chareyre[2], H. Zouali[2], S. Lesage[2], S. Girardin[3], B. The Hung[2], U. Zaehringer[5], P. Sansonnetti[3], J.P. Hugot[2], G. Thomas[1,2].* 1) INSERM U434, Paris, France; 2) Fondation Jean Dausset, Paris, France; 3) Institut Pasteur, Pathognie Microbienne Molculaire, Paris, France; 4) Institut Pasteur, Groupe 5 ans "Immunit Inne et Signalisation", Paris, France; 5) 5 Forschungszentrum Borstel, Zentrum fr Medizin und Biowissenschaften, Borstel, Germany.

Crohn's Disease (CD [OMIM266600]) is one of the main form of Chronic Inflammatory bowel Diseases affecting approximately a million of Northern Americans and Western Europeans. By positional cloning, we previously identified CARD15 (formerly called NOD2) as a susceptibility gene to this multifactorial disorder. Using transient transfection assays in human cells, we have now shown that CARD15 is implicated as a potent signal transducer sensing bacterial peptidoglycan (PG), but not pure lipopolysaccharide. Thus CARD15 may play a role in PG-induced proinflammatory signaling. Comprehensive mutational analysis of CARD15 had previously revealed a marked excess of variants among CD patients. The functional analysis of 32 such variants identified several levels of molecular defects that hinder PG-induced NF-kB activation. Patients were classified according to the NF-kB activation potential deduced from their CARD15 genotype. Patients with the lowest potential had the earliest age at onset, the highest frequency of granulomatous lesions and the most severe clinical course. Thus, CARD15-mediated susceptibility to CD may be due to a defective triggering of protective NF-kB activation by intracytoplasmic PG in infected cells. The inappropriate response of these cells may subsequently result in the diffuse activation of the NF-kB pathway in CD lesions by a CARD15-independent mechanism. Distinct CARD15 mutations have also been implicated in the dominantly inherited granulomatous disease, Blau syndrome. In the same functional assay, the corresponding mutations overactivate the NF-kB pathway in the absence of upstream signal. In this case, pathological inflammation may be caused by a diffuse constitutive NF-kB activation caused by the increased basal activity of CARD15.

Program Nr: 139 from 2002 ASHG Annual Meeting

**Genome-wide search in asthma and atopy patients identifies multiple loci of genome-wide significance.** *H. Hakonarson[1], E. Halapi[1], U.S. Bjornsdottir[2], R. Upmanyu[1], E. Soebech[1], D. Gislason[2], A.E. Adalsteinsdottir[1], G. Finnbogason[1], T. Gislason[2], M.L. Frigge[1], A. Kong[1], J.R. Gulcher[1], K. Stefansson[1]*. 1) Pulmonary Research, deCode Genetics, Inc, Reykjavik, Iceland; 2) Department of Allergy/Pulmonary Medicine, the National University Hospital, Reykjavik, Iceland.

**Background** Several studies have reported linkage or association of asthma to various candidate genes and loci. Although a few atopy genes have been mapped to a genome-wide significance using different parametric models, no asthma gene conferring high risk has been mapped. **Methods** We performed a genome-wide scan with 1,100 microsatellite markers using 241 extended Icelandic families with 837 asthma and atopy patients. The families were identified by cross-matching a list of asthma patients from the University Hospital with a genealogy database of the entire Icelandic population. Analysis was performed to examine linkage to asthma, (596 patients), asthma and/or atopy (837 patients), and atopy alone as measured by elevated IgE >70 IU/L (241 patients). In addition, linkage was examined in 24 families with glucocorticoid resistant asthma (88 patients). **Results** We detected linkage of asthma to chromosome (chr) 14q24 with an allele-sharing lod score of 4.00 (p= 8.7 x 10-6). A lod score of 4.7 was found on chr 5p using all 837 patients. Two separate loci were detected for the elevated IgE (atopy) phenotype on chr 3p and 11q with lod scores of 3.1 and 4.3, respectively. In addition, linkage analysis of 24 families with glucocorticoid resistant asthma uncovered a locus with a lod score of 2.6 on chr 18q. **Conclusions** These results provide evidence for a novel asthma susceptibility gene on chr 14q24, gene(s) for asthma and atopy on chr 5p, genes for atopy without asthma to chr 3p and 11q, respectively, whereas gene(s) for glucocorticoid resistant asthma localized to chr 18q. Studies are currently underway to examine epistasis between these genes.

Program Nr: 140 from 2002 ASHG Annual Meeting

**Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness.** *T.P. Keith[1], R.D. Little[1], J. Dupuis[1], R.G. Del Mastro[1], K. Falls[1], J. Simon[2], D. Torrey[1], K. Braunschweiger[1], A. Walsh[1], Z. Liu[1], B. Hayward[1], J. Dubois[1], S.P. Umland[2], R.W. Egan[2], F.M. Cuss[2], S. Rorke[3], J.B. Clough[3], J.W. Holloway[3], S.T. Holgate[3], P. Van Eerdewegh[1]*. 1) Human Genetics Dept., Genome Therapeutics Corp., Waltham, MA; 2) Schering-Plough Research Institute, Kenilworth, NJ; 3) Respiratory, Cell and Molecular Biology, Infection, Inflammation and Repair Research Division, School of Medicine, University of Southampton, Southampton General Hospital, Southampton, Hampshire, England.

To identify susceptibility gene(s) for asthma, we performed a genome wide scan on 460 Caucasian families and identified a locus on chromosome 20p13 linked to asthma (MLS = 2.94) and bronchial hyperresponsiveness (BHR, MLS = 3.93). Gene discovery efforts were focused in a 1-LOD-drop support interval. A physical map was developed across this 2.5 Mb region and selected BAC clones sequenced. Direct cDNA selection, cDNA library screening, RT-PCR, and public data mining were used to characterize genes in the region that were prioritized for association studies. Polymorphisms in 23 genes spanning this interval were identified through fluorescent SSCP and DNA sequencing. A survey of 135 polymorphisms identified the ADAM33 gene (A Disintegrin and Metalloprotease) as being significantly associated with asthma using case-control, transmission disequilibrium (TDT), and haplotype analyses ($p = 0.04$ to $p = 0.000003$). Interestingly, a quantitative trait locus for airway hyperresponsiveness (bhr1) has been mapped to a region on mouse chromosome 2, which is syntenic to human 20p13, strikingly close to the location for the mouse ortholog of ADAM33. ADAM proteins are membrane-anchored metalloproteases with diverse functions including the shedding of cell-surface proteins such as cytokines and cytokine receptors. A functional role for ADAM33 in airway remodeling and BHR is supported by abundant expression in human lung fibroblasts and bronchial smooth muscle, with expression notably absent from bronchial epithelial cells. Further characterization of ADAM33 should provide unique insights into the pathogenesis of this common disease.

Program Nr: 141 from 2002 ASHG Annual Meeting

**Linkage disequilibrium and haplotype diversity on chromosome 20p13 around the asthma susceptibility gene ADAM33.** *P. Van Eerdewegh[1], J. Dupuis[1], K. Falls[1], R. Little[1], B. Hayward[1], A. Bureau[1], R. Del Mastro[1], S.P. Umland[2], R.W. Egan[2], F.M. Cuss[2], S.T. Holgate[3], T.P. Keith[1].* 1) Human Genetics Dept., Genome Therapeutics Corp, Waltham, MA; 2) Schering-Plough Research Institute, Kenilworth, NJ; 3) Respiratory, Cell and Molecular Biology Division, School of Medicine, University of Southampton General Hospital, Southampton, Hampshire, England.

ADAM33 has been identified as an asthma and bronchial hyperresponsiveness (BHR) susceptibility gene using a positional cloning approach. Linkage analysis in affected sibpairs (LOD of 2.94 (asthma) and 3.93 (BHR)) was followed by association studies in the subset of families contributing to the linkage evidence using case-control and transmission disequilibrium (TDT) tests (p=0.04 to p=0.000003). The sample consisted of 460 Caucasian families from Southampton (UK) and various collection sites in the US with 217 unrelated controls, matched for ethnicity and country of origin. We measured linkage disequilibrium (LD) in the region around ADAM33 by two measures: Delta and D', with the former related to the association evidence and the latter to the haplotype diversity. The pattern of Delta differed between the US and UK populations, and SNPs in strong LD by this measure formed groups of non-adjacent SNPs with similar level of association with the asthma phenotype. Statistical haplotyping of 80 SNPs in 7 genes spanning 247 kb around ADAM33 was performed to elucidate the haplotype diversity and block structure of LD in that region of chromosome 20. The low diversity illustrated by the small number of haplotypes with an estimated frequency of 1% or larger is in contrast to the more complex picture found with the measure of LD (Delta) that is relevant to the identification of a causal variant within a gene in a case-control association study. Detection of an association is critically dependent on both the allele frequency and the non-monotonic nature of LD as a function of distance. This is illustrated by measuring the pairwise LD among the 37 SNPs typed in ADAM33 and the association of specific SNPs and haplotypes with the asthma phenotype in this study.

Program Nr: 142 from 2002 ASHG Annual Meeting

**AIRE MODULATES IFN-g EXPRESSION IN T LYMPHOCYTES.** *P. Cavadini[1], D. Moratto[1], A. Sica[2], M.C. Rosatelli[3], L.D. Notarangelo[1], R. Badolato[1]*. 1) Pediatrics, Univ. of Brescia, Italy; 2) Dept of Immunology and Cell Biology, Mario Negri, Milano, Italy; 3) Istituto di Ricerca sulle Talassemie, Univ. of Cagliari, Italy.

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autosomal recessive disorder with higher prevalence in some isolated populations. It is characterized by autoimmune-mediated failure of multiple endocrine glands, chronic or recurrent mucocutaneous candidiasis, and dystrophy of several ectodermal tissues. APECED is a monogenic disorder caused by mutations in the autoimmune regulator gene (AIRE). The encoded protein AIRE has transcriptional transactivating properties and is able to form multimers that bind the ATTGGTTA motif, suggesting that AIRE is a DNA binding protein. To investigate the function of this protein in the mechanism of maturation of T lymphocytes we have analyzed AIRE expression in normal leukocytes and cytokine production by PBMC separated from normal subjects or APECED patients. By RT-PCR we have found that AIRE is expressed in EBV transformed B cell lines and in T lymphocytes, both resting as well as in CD3 or PHA activated cells. Analysis of IFN-g release from purified CD4+ lymphocytes activated with immobilized anti-CD3 revealed a consistent defect in IFN-g release suggesting that the defect in activation of IFN-g gene is intrinsic to T lymphocytes. Because we found a sequence in the promoter region of IFN-g highly homologous to the putative binding consensus sequence for AIRE we hypothesized that AIRE is able to bind this region. To test this hypothesis we have cotransfected transiently COS cells with a plasmid expressing AIRE (pCDNA-AIRE) and a plasmid in which the -2.7Kb of IFN-g promoter is fused with a b-Galactosidase cDNA (pIFN-2.7). Preliminary results showed a ~ 40 times induction of b-Galactosidase activity in the cells cotransfected with pCDNA-AIRE and a pIFN-2.7 compared with the cells costransfected with the parental vector (pCDNA) and pIFN-2.7. We are also performing gel shift analysis to identify the AIRE binding site in IFN-g promoter. These results suggest that development of autoimmune disorders in APECED patients may arise from deregulation of cytokine expression by lymphocytes.

Program Nr: 143 from 2002 ASHG Annual Meeting

**Genetic Polymorphism in the pol Gene and Altered p27 Gene-a Secondary Mechanism Involved in HAART Resistant in Human Immunodeficiency Virus (HIV-1).** *E.M. McGhee, W.L. Holzemer, D.J. Wantland.* Dept CHS, Univ California, San Francisco, San Francisco, CA.

Genetic polymorphism mutations contribute to highly active antiretroviral therapy (HAART) failure in human immunodeficiency virus type1 (HIV-1). Target mutations within the pol gene, that encodes the viral protease (PR), reverse transcriptase (RT), and integrase (I) may not be the sole abnormality that confer resistant to HIV-1 therapy. We speculate that secondary mechanisms and mutations in other genes (cell cycle regulatory p27), other than the drug target may influence failed therapy in infected patients. For this purpose, p27 cell cycle gene was analyzed by flow cytometry. We analyzed genotype profiles of HIV-1 patients undergoing HAART (N=30). The base sequence changes were determine by polymerase chain reaction (PCR), using HIV-1 patients cellular RNA extract. Our data show that the genotype profiles for the pol gene, in HIV-1 patients exhibited a spectrum of mutations in the RT and PR genes at multiple codon sites. We calculated the mutation frequency and used SPSS.10 to analyze the data. Our preliminary data show a complex mutation distribution in the RT and PR genes. We report common mutations at 13 different codon sites; [Codon {mutation %}]: RT gene, 122(60%), 162(23%), 174(20%), 200(33%), 211(46%), 214(73%), and 245(46%). PR gene, 37 (80%), 62(20%), 64(20%), 69(13%), 72(13%), 93(13%). Our cell cycle data show that in control samples 95% of cells were in G1, 1% in G2/M, and 2% in S. In the HIV-1 infected cells 90% of cells were in G1, 1% in G2/M, and 9% in S. These preliminary results show that there is significant common genetic polymorphism in the RT and PR genes, and that the elevated S-phase in the cell cycle indicates that p27 may be involved in HIV-I therapy resistant. Overall, the data show that there are secondary events involved in HAART failure, indicating nuclear changes, which may also include plasma and mitochondrial membrane perturbations. Further studies on gene analysis will help us to better understand cellular events and mechanisms involved in HIV-1 infected cells that will lead to better therapy.

Program Nr: 144 from 2002 ASHG Annual Meeting

**Primary Ciliary Dyskinesia : Characterization and mutation scan of DNAH17 gene on chromosome 17q coding for an axonemal heavy chain dynein.** *C. Albrecht[1], C. Gehrig[1], G. Duriaux Saïl[1], C. Rossier[1], C. Gumy[1], M. Künzli[2], S.E. Antonarakis[1], C. DeLozier-Blanchet[1], J.-L. Blouin[1]*. 1) Medical Genetics, University Medical School and University Hospitals, Geneva, Switzerland; 2) Kantonsspital, Aarau, Switzerland.

Primary Ciliary Dyskinesia (PCD; autosomal recessive; incidence 1/20,000), affects respiratory cilia and spermatozoa flagella motility. Dysmotility to complete immobility causes recurrent bronchial infections, bronchiectasis, sinusitis, rhinitis, male subfertility. Situs inversus is observed in 50% of cases (Kartagener Syndrome). Our genome-wide linkage analysis (Blouin et al., 2000) which showed extensive genetic locus heterogeneity, indicated several potentially linked genomic intervals for PCD to which dynein genes have mapped (3p,5p,16p,17q). Dynein arms are found to be defective or missing in 50% of PCD patients. The numerous dyneins are excellent candidates for the disease since, todate, mutations in 3 genes coding for dyneins DNAI1, DNAH11 and DNAH5 have been detected in a few PCD patients. The DNAH17 gene, which maps on chromosome 17q25, a locus indicated by linkage analysis, encodes a protein that is highly homologous to the Sea Urchin axonemal b-heavy chain outer arm dynein . We report here the full-length genomic organisation, transcript and predicted protein sequences, and mutations scans of the gene DNAH17 in PCD patients. The DNAH17 gene spans 178 Kb of genomic sequence with a transcript of 13.4 Kb (79 exons) coding for a protein of predicted size of 4470 residues. We initiated a mutation scan in all exons, on 4 pedigrees of our collection of multiplex families showing haplotypes compatible with complete linkage between PCD and the locus of DNAH17 gene on chromosome 17q23-25 (interval DS17S1301-D17S928). Thusfar, we have identified a non-sense mutation (Y522X) in 2 patients from one family. Several missense nucleotide variants were also detected for which the potential pathogenicity is now under study. These data provide supportive evidence that mutations in the DNAH17 gene contribute to the pathology of PCD. (Supported by the Swiss National Funds and the Carvajal Foundation of Geneva).

Program Nr: 145 from 2002 ASHG Annual Meeting

**Association study of multiple sclerosis in Portuguese patients: whole genome screen using 6000 microsatellite markers and a DNA pooling strategy.** *J. Pinto-Basto[1,2], M. Santos[1], M.E. Rio[3], A. Valença[4], J. Sequeiros[1,5], P. Maciel[1,5]*. 1) UnIGENe, IBMC, Porto, Portugal; 2) IGM, Porto, Portugal; 3) Serv. Neurol., Hosp. S.João, Porto, Portugal; 4) Serv. Neurol., Hosp. Força Aérea, Lisboa, Portugal; 5) Depto. Est. Pop., ICBAS-UP, Porto, Portugal.

Multiple sclerosis (MS) is a chronic demyelinating disease of the CNS that affects 1:500 European young adults. We performed a population-based association study using a sample of 183 unrelated MS patients (129 females), with an average age of 36.9 (17-64). All patients had definite MS diagnosis according to the Poser criteria; 24 were classified as having the primary progressive form of the disease, and the remaining had a relapsing-remitting or secondary progressive presentation. Age at onset ranged from 6 to 55 (average: 27.9) years. We also studied 183 controls, individually matched by sex, age and region of origin. For the pooling procedure, DNA concentration was measured twice with a spectrophotometer, diluted to a concentration of 60 ng/ml, which was confirmed using the picogreen system; and then further diluted to a final concentration of 25 ng/ml. Two DNA pools were prepared from DNAs of cases and controls, respectively. We then studied 6000 microsatellite markers genomewide, with an average distance of 0.5 cM. Two PCRs using fluorescent-labelled primers were performed for each marker and the products were electrophoresed twice in a capillary sequencer; amplification was successful for 5339 markers. To determine allele frequencies, we weighted each allele peak height to the sum of peak heights. Allele frequencies of cases and controls were compared for each marker using the $c^2$ test. We ranked the markers by their p-value and then chose 46 out of those with a p-value $<10^{-3}$ for confirmation. The most promising regions identified in this study were 18p, 11p, 15q, 10p, 5q, 7q, and 6p. Replication of previous findings of association with markers in the HLA region suggests that this methodology is appropriate to detect *loci* associated to MS. Comparison of the results obtained in this and other populations should contribute to the identification of the relevant *loci* for susceptibility to MS.

Program Nr: 146 from 2002 ASHG Annual Meeting

**Haplotypes of the *RET* proto-oncogene modify risk of Hirschsprung disease in Italian patients.** *I. Ceccherini[1], P. Griseri[1], F. Lantieri[1], M. Sancandi[1], B. Pesce[1], G. Patrone[1], F. Puppo[1], M. Lerone[1], J. Osinga[2], R. Hofstra[2], G. Martucciello[3], R. Ravazzolo[1,4], M. Devoto[4,5]*. 1) Lab Genetica Molecolare, Inst Gaslini, Genova, Italy; 2) Dept Medical Genetics, Univ Groningen, NL; 3) Dept Pediatric Surgery, Inst Gaslini, Genova, I; 4) Dept Oncology, Biology and Genetics, Univ Genova, I; 5) Dept Research, Nemours Children's Clinic, Wilmington, DE, USA.

Hirschsprung disease (HSCR) is a congenital disorder of the enteric nervous system affecting intestinal motility. A complex model of inheritance is sustained by high proportion of sporadic cases, variable expressivity, incomplete penetrance and recent mapping of novel HSCR loci. Loss of function RET mutations account for 50% of familial and 10-20% of sporadic cases. Moreover, several common RET polymorphisms have been reported to show allelic association with HSCR. To identify RET haplotypes associated with different risks of HSCR development, we have genotyped 99 Italian sporadic patients and 85 population matched controls for 10 SNPs at the RET locus. Markers span 53.3 Kb, from the basal promoter to the distal end of the 3'UTR. A comparison of the reconstructed haplotypes in HSCR and controls has allowed us to focus on two particular RET haplotypes. One is under-represented in HSCR (0.5% vs 8.5%; $P<0.00023$) and displays non random trasmission from healthy carrier parents to affected sibs (only 1 transmission to an affected child from a total of 27 heterozygous parents of Italian and Dutch origin; $P<0.000002$). We hypothesized that this haplotype, which is conserved at the 3'end of the gene, confers a protective effect against HSCR development, probably due to disregulation of RET isoforms expression. The second haplotype is over-represented in HSCR (62% vs 22%), conserved at the 5'end of the gene and believed to have a predisposing effect. The co-presence, in this haplotype, of the A allele of SNP2 and of a specific combination of alleles of two novel SNPs, detected at -1nt and -5nt from transcription start site, suggests RET expression may be affected. Identification of functional variants in linkage disequilibrium with these two haplotypes is in progress.

Program Nr: 147 from 2002 ASHG Annual Meeting

**Transmission disequilibrium of a conservative SNP(135G>A, exon 2) in sporadic and familial Hirschsprung disease cases lacking RET proto-oncogene mutation.** *J. Amiel[1], L. de Pontual[1], A. Pelet[1], T. Attié[1], R. Salomon[1], F. Clerget[2], C. Mugnier[1], F. Matsuda[3], M. Lathrop[3], A. Munnich[1], J. Feingold[1], S. Lyonnet[1]*. 1) INSERM U-393, Hopital Necker, Paris; 2) INSERM U-535, Bicetre; 3) CNG, Evry, France.

HSCR is a frequent congenital malformation of the hindgut regarded as a model for the study of multigenic inheritance. Indeed, while a significant proportion of sporadic and familial cases have been ascribed to mutations of the RET proto-oncogene (15% and 50% respectively), two other susceptibility loci have been recently mapped to chromosomes 3p21 and 19q12 respectively in a non-parametric sib-pair linkage study. Therefore, a 3 genes model accounts both for the observed incidence of HSCR in the general population and for the recurrence risk in sibs. However, the low rate of RET gene mutations remains surprising especially as almost all familial HSCR cases were consistent with linkage to the RET locus. We further studied the RET gene coding sequence in a series of sporadic and familial HSCR patients, and focused on patients with no RET gene mutation identified by direct DNA sequencing (N=50). We performed a transmission disequilibrium test (TDT) including sporadic cases (n=28) and the first sib in sibships with two or more affected sibs (n=22). For a conservative polymorphism in RET exon 2 (135G>A, A45A), we analysed the 3 informative types of mating namely: A/G x A/A (n=14), A/G x A/G (n=18), and A/A x A/G (n=8). The observed transmission differed very significantly from the theoretical distribution with an excess of transmission of the 135A allele (c2=23.44, p<10-5). When considering all affected sibs (linkage analysis), the difference remained highly significant (c2=37.78, p<10-6). Furthermore, the 135A nucleotide was found in various haplotypic combinations with other 3' RET exonic polymorphisms. The question of whether the 135A allele is a hypomorphic disease causing allele or unravels linkage disequilibrium with an unknown located nearby mutation remains unanswered. However, these data already suggest that frequent low-penetrance allele(s) at the RET locus account for the low rate of mutation detection in HSCR.

Program Nr: 148 from 2002 ASHG Annual Meeting

**Genome-wide association study in Mennonites identifies multiple genes for oligogenic Hirschsprung disease.**

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Genetic studies of Hirschsprung disease (HSCR) have identified seven genes with associated mutations, however, individual mutations are neither necessary nor sufficient to cause clinical disease. We conducted a genome-wide association study in a Mennonite isolate and created appropriate mouse models to demonstrate that genetic interaction between mutations in the receptor tyrosine kinase (RTK) RET and the G-protein coupled receptor (GPCR) EDNRB is an underlying mechanism for this complex disorder.

We genotyped a combination of 2,083 microsatellites and single nucleotide polymorphisms (SNP) in 43 family trios, each consisting of one affected child and two parents. EDNRB on 13q22 is known to be a susceptibility gene in Mennonites. The marker genotypes were analyzed using a new multipoint linkage disequilibrium method (MLD), which searches for association arising from common ancestry for a mutation. We observed MLD lod scores of 55.60, 5.60 and 3.01 with genetic markers at 13q22, 10q11.2 and 16q23.3, respectively. We demonstrate the chromosome 10 locus to be RET, by fine-scale linkage disequilibrium analyses using 32 SNPs. A genetic interaction between RET and EDNRB can be shown by significant ($P<0.0001$) joint transmission, as well as by generating mouse intercrosses between Ret knockout mice and mice harboring the hypomorphic piebald Ednrb mutation. The penetrance of the two locus genotypes in Mennonites varies between 0 and 100%. Thus, this study comprises one of the first reports of a genome-wide association scan leading to identification of susceptibility variants in two genes resulting in complex inheritance.

Program Nr: 149 from 2002 ASHG Annual Meeting

***FOXD3* promoter variants co-segregate with generalized vitiligo in chromosome 1p-linked families.** *A. Alkhateeb[1], P.R. Fain[1], A. Fox[1], D.C. Bennett[2], R.A. Spritz[1].* 1) Human Medical Genetics Prog, Univ Colorado Heath Sci Ctr, Denver, CO; 2) Dept Anat Devel Biol, St George's Hosp Med Sch, London, UK.

Generalized vitiligo is a common, acquired autoimmune disorder in which patchy loss of skin and hair pigmentation results from a loss of pigment-forming melanocytes from the involved areas. By genomic mapping of multiplex families with vitiligo, we localized a vitiligo susceptibility locus, *AIS1*, to chromosome 1p31.3-p32.2. We have examined a promising biological positional candidate gene, *FOXD3*, located within the 7.4-Mb *AIS1* genetic interval. FOXD3 is a forkhead/winged helix transcription factor that regulates vertebrate embryogenesis, with a major role in early specification, segregation, and differentiation of the neural crest. *FOXD3* expression is repressed in melanoblasts, as its expression represses melanogenesis. We sequenced *FOXD3* in a large chromosome 1p-linked family and identified a sequence variant in the *FOXD3* promoter that co-segregates with the linked haplotype. This variant occurs within a transcriptional motif originally found in an *EGFR* enhancer and was not found in 200 control chromosomes. We next sequenced *FOXD3* in 5 additional multiplex vitiligo families that showed evidence of linkage to the *AIS1* region, and found two more families with sequence variants in the *FOXD3* promoter that co-segregated with the vitiligo phenotype, one in a motif virtually identical to that containing the variant in the first family. These data suggest that *FOXD3* promoter variants may predispose to vitiligo in some families, possibly by de-repressing *FOXD3* expression and thereby predisposing to melanocyte cell death and autoimmune response against melanocyte components. We are currently carrying out functional analyses of these variant *FOXD3* promoters to assess their transcriptional activity in transfected melanocytes.

Program Nr: 150 from 2002 ASHG Annual Meeting

**Identification of a susceptibility gene for uric acid kidney stones.** *F. Gianfrancesco[1,2], T. Esposito[1], M.N. Ombra[1], P. Forabosco[1], G. Maninchedda[1], M. Fattorini[1], S. Casula[1], S. Vaccargiu[1], G. Casu[1], F. Cardia[2], I. Deiana[3], M. Falchi[1], M. Pirastu[1,2]*. 1) Istituto di Genetica delle Popolazioni, CNR, Alghero, SS, Italy; 2) Shardna, Cagliari, Italy; 3) ASL 4, Lanusei,NU,Italy.

We previously identified a susceptibility locus for uric acid nephrolithiasis (UAN), a common multifactorial disorder accounting for 20% of all kidney stones, on chromosome 10q21-q22 in patients originating from a small isolated village of Sardinia (Ombra et al. 2001). In order to refine the critical region and identify the susceptibility gene we extended our analysis to a larger sample of affected subjects collected from the same isolate. By alignment of partial sequences present in different databases we generated a consensus genomic sequence and identified 36 new SNPs in the 1.2 Mb critical region. Through LD-mapping and haplotype analyses we refined the critical region to a haplotype LD-block of about 200 kb that was more prevalent in affected cases than controls. In unrelated controls this haplotype was associated with low UpH, which is an important risk factor for UAN. By sequence analyses and database searches, we determined that a novel gene overlaps with this interval. Molecular characterization performed by EST analysis, RT-PCR and RACE strategies, revealed that it was splitted in 15 exons spanning a region of about 280 kb generating by alternative splicing at least four proteins of 407, 333, 462 and 216 amino acids. Interestingly, the last one was completely included in the LD-block associated with UAN. Computer-assisted analysis of this isoform revealed at least a membrane-spanning domain, and several N and O-glycosylation consensus sites at N-termini suggesting that it could be an integral membrane protein. Based on the hypothesis that this protein of 216 aa may assume a role in uric acid nephrolitiasis, mutational analysis revealed a coding single nucleotide variant which causes a missense in the exon 4 (Ala62Thr) in strong association with UAN (P=0.0096). It was interesting to note that the presence of the Thr62 induced a dramatic change of the secondary structure (alpha elic loop) of the protein suggesting a mechanism for a putative biochemical effect.

Program Nr: 151 from 2002 ASHG Annual Meeting

**The physical map of the multiple sclerosis susceptibility locus on chromosome 17q22-24 exposes blocks of segmental duplication.** *J. Saarela[1,2], D. Chen[2], W.S. Chi[2,4], E. Eichler[3], S. Finnila[2], A. Jokiaho[2], M. Eeva[2], A. Palotie[2,4], L. Peltonen[1,2]*. 1) Molecular Medicine, National Public Health Inst, Helsinki, Finland; 2) Human Genetics, UCLA, LA, CA; 3) Genetics, Case Western Univ, Cleveland, OH; 4) Pathology, UCLA, LA, CA.

MS is one of the most common neurological diseases of young adults. We have used a study sample of 26 multiplex MS-families, most originating from an internal subisolate of Finland and established linkage to chromosomes 5p, 6p, 17q and 18q, all loci also identified in other populations. The locus on chromosome 17q gave the best initial evidence for linkage and was selected the first focus for positional cloning of the putative MS gene. This region has been a challenge for positional cloning due to the numerous genomic rearrangements and duplications. In order to resolve map discrepancies in databases and thus improve the map of the region, we combined information from FISH, Radiation Hybrid mapping and PCR ordering of physical clones. 193 BACs were further analysed computationally using a segmental duplication database (SDD) which combines both public and private sequencing resources to identify blocks of recent genomic duplication (Bailey et al., Science, in press). 17 BACs mapping to 4 distinct regions within the interval showed evidence for large blocks (10-100 kb in length) of duplication with >95% sequence identity. This computational data was compared to our laboratory-experimental evidence for duplications. So far 4 of these 11 BACs have been tested by FISH and RH mapping (determined by the number of positive clones in the RH panel). Interestingly, all of these four BACs tested show evidence for duplication both in FISH and RH mapping. Using a population subisolate and combined power of linkage, linkage disequilibrium and monitoring for shared haplotypes, we have restricted the MS locus on to less than 3.9 Mb region. This region is flanked by duplicated/triplicated segments. So far we have genotyped 55 SNPs in 28 multiplex families and 417 trios. Two of the SNPs located in the middle of the shared haplotype region show significant association (p=0.000005 and p=0.000018, respectively) to MS using the HRR test.

Program Nr: 152 from 2002 ASHG Annual Meeting

**Full Genome Screen for Alzheimer Disease: Stage III Analysis.** *J. Williams[1], P. Holmans[2], D. Turic[1], P. Hollingworth[1], M. Hamshere[1], F. Wavrant De-Vrieze[4], R. Crook[4], D. Crompton[4], H. Marshall[5], A. Meyer[5], J. Booth[5], J.C. Morris[6], J. Powell[3], S. Lovestone[3], J. Hardy[4], A. Goate[5,7], L. Jones[1], M.C. O'Donovan[1], M.J. Owen[1]*. 1) Psychological Medicine, UWCM, Cardiff, UK; 2) MRC Biostatistics Unit, Institute of Public Health, University Forvie Site, Cambridge, UK; 3) IOP, De Crespigny Park, London, UK; 4) Lab of Neurogenetics, Birdsall Building, Mayo Clinic Jacksonville, Jacksonville, Florida; 5) Dept of Psychiatry, Washington University School of Medicine, St Louis, Missouri; 6) Dept of Neurology, Washington University School of Medicine,St Louis, Missouri; 7) Dept of Genetics, Washington University School of Medicine, St Louis, Missouri.

We performed a two-stage genome screen to search for novel risk factors for late-onset Alzheimer disease (AD). The first stage involved genotyping 292 affected sibling pairs using 237 markers spaced at approximately 20 cM intervals throughout the genome. In the second stage, we genotyped 451 affected sibling pairs (ASPs) with an additional 91 markers, in the 16 regions where the multipoint LOD score was greater than 1 in stage I. Ten regions maintained LOD scores in excess of 1 in stage II, on chromosomes 1 (peak B), 5, 6, 9 (peaks A and B), 10, 12, 19, 21, and X. Our strongest evidence for linkage was on chromosome 10, where we obtained a peak multipoint LOD score (MLS) of 3.9. The linked region on chromosome 10 spans approximately 44 cM from D10S1426 (59 cM) to D10S2327 (103 cM). In the Stage 3 analysis we tested for linkage to complex AD phenotypes using co-variate linkage analysis including factors such as age of onset, sex, linkage status (APOE & chromosome 10 loci), rate of decline, psychosis, agitation, aggression and depression. We observed significant linkage (LOD>4) to age of onset in AD cases on chromosome 21 and found other areas which show evidence of linkage to complex AD phenotypes.

Program Nr: 153 from 2002 ASHG Annual Meeting

**Polymorphisms of LG72 (on 13q33) associated with Bipolar disorder by TDT and partition of linkage evidence.**

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Linkage evidence suggests chromosome 13 (13q32-33) contains both Bipolar and Schizophrenia susceptibility genes. In this chromosomal region I. Chumakov of Genset Corporation recently presented data showing association with Schizophrenia of polymorphisms in the gene LG72. We studied two Bipolar pedigree series, CNG Pedigrees (where linkage to illness had been previously reported at 13q32-33) with 85 samples from 22 multiplex families, and NIMH Genetics Initiative Pedigrees (without significant linkage) with 478 samples from 97 families. Six single nucleotide polymorphisms (SNPs) at the LG72 locus were selected from public SNP databases, which covered a ~41 kb region containing the whole putative cDNA sequence, and were spaced at 4-13 kb intervals. We performed transmission disequilibrium test (TDT) and haplotype analysis, as these six markers were in linkage disequilibrium with each other (D' = 0.59- 0.95) except for between the two at the 5 and 3 extremes. In the CNG data set, three individual markers were in linkage disequilibrium with illness ($P < 0.0009$, 0.007, 0.009), as were several haplotypes. In analysis of partitioning of the evidence for linkage according to genotype, several markers were positive, with the most significant showing $P < 0.00026$. Decay of haplotype sharing (DHS) analysis gave a location estimate including LG72 in its 99% confidence interval. In the NIMH data set, nominally significant transmission disequilibrium was also found for several of the same haplotypes as in the CNG set. These data are consistent with a susceptibility variant for Bipolar illness in the vicinity of the LG72 gene.

Program Nr: 154 from 2002 ASHG Annual Meeting

**Identification of a high risk haplotype in the dystrobrevin binding protein 1 (DTNBP1) gene from the Irish Study of High Density Schizophrenia Families.** *B.P. Riley[1], E. van den Oord[1], Y. Jiang[1], P.F. Sullivan[1], X. Chen[1], F.A. O'Neill[2], D. Walsh[3], K.S. Kendler[1]*. 1) Dept Psychiatry/Human Gen, Virginia Commonwealth Univ, Richmond, VA; 2) Department of Psychiatry, Queens University, Belfast,; 3) The Health Research Board, Dublin, Ireland.

We have previously reported linkage of schizophrenia to chromosome 6p24-p22 in a sample of 270 multiply-affected families from Ireland. Initial replications and metanalyses supported these linkage findings. Linkage disequilibrium analysis of a dense microsatellite map of the region showed association in the interval D6S260-D6S1676. Single nucleotide polymorphisms (SNPs) in one known gene (JMJ) and an EST cluster in the interval, and an additional known gene (MIR) centromeric of D6S1676 were assessed for association. Markers in the EST cluster show association, and markers in the known genes do not. We subsequently characterized the EST cluster as the dystrobrevin binding protein 1 (DTNBP1) gene, the human ortholog of murine dysbindin. Markers within DTNBP1 give evidence of association by a variety of tests. The most significant single markers are p1635 (in intron 4, p=0.0001 by TRANSMIT) and p1320 (in intron 3, p=0.0005, by PDT). As a follow-up to the report by Straub et al (2002), we have identified a haplotype block defined by 8 of the SNPs used in these analyses and a specific schizophrenia high risk haplotype within this block. The haplotype block corresponds to the 5-most exons of DTNBP1 and is approximately 30 Kb in size. In our schizophrenia families, we observe excess transmission of the haplotype (p=0.002, by PDT), which is found in 55 affected offspring in 38 (14%) of our 270 families. Cladistic analysis identifies the relationships between the 7 common haplotypes within this block that account for 94% of founders in the sample. Analysis of the geographical origin of these families shows no evidence for a specific founder effect within Ireland.

Program Nr: 155 from 2002 ASHG Annual Meeting

**Identification of a genetic risk factor on chromosome 10 for diabetes mellitus in the Framingham Heart Study population.** *A.G. Herbert[1], J.B. Meigs[2], P. Wilson[3], C. Panhuysen[3], L.A. Cupples[4], S. Karomohamed[1], J. Liu[1], J. Volcjak[1] and Framingham Heart Study.* 1) Neurology, Boston University School of Medicine, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Department of Medicine, Boston University School of Medicine, Boston, MA; 4) Department of Biostatistics, Boston University School of Medicine, Boston, MA.

The Framingham Heart Study (FHS) was initiated in 1948 to identify factors and characteristics contributing to cardiovascular disease. Epidemiological investigations have led to the identification of a number of risk factors including environmental, lifestyle and clinical measures. Genetic studies using the two generations of FHS participants have also been conducted, including genetic risk factors for type 2 diabetes. A number of measures representing sub-phenotypes for type 2 diabetes such as fasting blood glucose, fasting insulin and glycated hemoglobin (HbA1C) have a heritability of between 30 and 40% in our cohort. In a genome scan of 330 pedigrees with 1702 subjects genotyped, we found a region on chromosome 10 likely to harbor a gene that influences fasting plasma glucose. This peak covers about 20 megabases of the chromosome and has a maximum LOD score of 2.1. To narrow this region further, fine mapping of chromosome 10 was conducted by placing microsatellite markers in candidate genes and testing for association between particular alleles and trait values firstly in families (n=1078), and then replicating the results in a set of unrelated individuals (n=1811). Additional mapping was completed using SNP polymorphisms assayed by MALDI TOF mass spectrometry. Using this strategy, we found by regression analysis(adjusting for known potential confounders)association in unrelated individuals between fasting glucose ($p<.002$) and HbA1C ($p<.0001$) trait values and a 54 kb region of chromosome 10. These results were replicated using the family based association test FBAT (glucose, $p<0.02$, HbA1C, $p<0.02$).Further, we show association with Diabetes Mellitus ($p<.02$) in unrelated individuals.

Program Nr: 156 from 2002 ASHG Annual Meeting

**Frizzled-4 Mutations Disrupt Retinal Angiogenesis in Familial Exudative Vitreoretinopathy (FEVR).** *M.L.E. MacDonald[1], J. Robitaille[2], A. Kaykas[3], L. Sheldahl[3], M.-P. Dube[1], D. Guernsey[2], B. Zheng[2], L. Siebert[4], A. Hoskin-Mott[2], S.N. Pimstone[1], B.S. Shastry[5], R.T. Moon[3], M.R. Hayden[1,6], Y.P. Goldberg[1,6], M.E. Samuels[1].* 1) Xenon Genetics, Inc., Burnaby, BC, Canada; 2) Dalhousie Univ, Halifax, NS, Canada; 3) Howard Hughes Med Inst, Univ of Washington, Seattle, WA; 4) Univ of Western Ontario, London, ON, Canada; 5) Oakland Univ, Rochester, MI; 6) Univ of British Columbia, Vancouver, BC, Canada.

Familial exudative vitreoretinopathy (FEVR) is a hereditary ocular disorder characterized by a failure of peripheral retinal vascularization. This may lead to neovascularization followed by retinal detachment and blindness. There are three identified FEVR loci, at 11q (EVR1), Xp (EVR2) and 11p (EVR3). We ascertained a large multigenerational family segregating FEVR, and confirmed linkage to the EVR1 locus. We further refined the disease locus to a small genomic region spanning about 1.5 MBp. The interval contains the frizzled-4 gene (FZD4), a member of a family of Wnt receptors. Upon sequencing genes within the region, we found a novel deletion of two amino acids in FZD4, which segregated completely with affected individuals in the family. A second mutation in FZD4, a two base pair deletion causing a frame shift, was detected in an additional unrelated FEVR family. Neither mutation was observed in 153 control chromosomes. The deleterious effects of these mutations were verified biochemically. Injection into Xenopus laevis embryos revealed that both mutant frizzled-4 proteins were defective in activating calcium/calmodulin-dependent protein kinase II (CAMKII) and protein kinase C (PKC), components of the Wnt/Ca2+ signaling pathway. One of the mutants also appeared defective in subcellular trafficking. These findings support a function for frizzled-4 in retinal angiogenesis and establish the first association between a FZD gene and a human genetic disorder. The clinical presentation of FEVR is identical to that of retinopathy of prematurity (ROP), suggesting that Wnt pathway genes may play a role in the development of both diseases. This work strengthens a body of evidence linking Wnt proteins and their receptors with processes of angiogenesis.

Program Nr: 157 from 2002 ASHG Annual Meeting

**Transgenic Mice with Partial Lipodystrophy due to expression of Lamin A or C cDNA containing a Dunnigan-specific mutation.** *K. Edgemon[1, 2], M. Haluzik[1], D.A. Cutler[1,2,3], M.J. Zarnowski[1], O. Gavrilova[1], C. Londos[1], M.L. Reitman[1,4].* 1) NIDDK/NIH, Bethesda, MD; 2) Genetics Program, George Washington Univ., Washington DC; 3) NCI/NIH Frederick, MD; 4) Merck Research Laboratories, Rahway, NJ.

Familial Partial Lipodystrophy - Dunnigan's variety (FPLD) is an autosomal dominant disorder characterized by loss of subcutaneous fat from the limbs and trunk around the time of puberty and increased fat deposition around the face and neck. Many FPLD individuals develop insulin resistance, diabetes, and hyperlipidemia. FPLD is caused by certain missense mutations in the Lamin A/C gene (*LMNA*) in the region corresponding to the C-terminal globular domain. *LMNA* produces two major splice variants that differ only at their 3'ends (Lamin A and Lamin C). Both messenger RNAs encode intermediate filament proteins that provide a large portion of the structural scaffolding at the inner surface of the nuclear membrane. In order to characterize the nature of the biological defect, we have developed transgenic mice that express human Lamin A or Lamin C containing the R482Q mutation, which causes FPLD. Expression of these cDNAs is controlled by the adipocyte-selective aP2 promoter/enhancer. Northern blot analysis demonstrated transgene expression in all white and brown adipose depots tested, with trace expression in cardiac and skeletal muscle. The mutLMNA mice exhibit a general decrease in white and brown adipose tissue weight and increased liver steatosis, as compared to wild type littermates. This phenotype was present in all ages studied (17 weeks, 31 weeks, and 13 months), in both males and females, whether on a high-fat, low-fat, or standard chow diet. The mutLMNA mice also develop insulin resistance. Hyperinsulinemic euglycemic clamps demonstrated that both liver and muscle are insulin resistant. Some groups showed a trend toward increased serum triglycerides and serum glucose levels. The lipodystrophic phenotype was caused by expression of either mutated Lamin A or C. These mice are a tool for increasing our understanding of Dunnigan's lipodystrophy caused by Lamin A/C mutations.

Program Nr: 158 from 2002 ASHG Annual Meeting

**Partial phenotypic correction of the 70kDa Peroxisomal Membrane Protein (PMP70) deficient mouse by making it PPAR**a **deficient.** *G. Jimenez-Sanchez[1], I. Silva-Zolezzi[1], G. Thomas[2], D.S. Millington[3], D. Valle[1]*. 1) Howard Hughes Medical Institute and Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Duke University Medical Center, Durham, NC.

PMP70 is an ABC transporter in the peroxisomal membrane. To better understand its function and relationship to human disease, we produced *PMP70*knockout mice (*PMP70-/-*) by standard gene targeting methods. These mice are viable, and exhibit impaired peroxisomal b-oxidation of a-methyl branched very long chain fatty acids (ameVLCFA) (phytanic, pristanic) and a set of metabolic abnormalities consistent with excessive activation of PPARa including reduced hepatic glycogen, massive dicarboxylic aciduria, dramatic reduction in plasma carnitine and increased expression of peroxisomal acyl CoA oxidase (*ACOX*), thiolase (*ACAA1*), mitochondrial medium chain Acyl CoA dehydrogenase (*MCAD*), and w-hydroxylase *CYP4A1* in the ER. a-meVLCFA are known ligands of PPARa and our results are consistent with a model in which inappropriate accumulation of these compounds leads to excessive activation of PPARa. To test this hypothesis, we produced *PMP70/PPAR*a double deficient mouse by breeding the respective single knockout mice. The double homozygotes survive to age 3-4 weeks. Initial analysis of these animals shows correction of the unsaturated dicarboxylic aciduria and normalization of expression of *ACAA1*, *ACOX* and *MCAD*. Glycogen studies are in progress. We conclude that a major component of the *PMP70-/-* phenotype is due to inappropriate activation of PPARa and speculate that the relevant human phenotype may include unexplained dicarboxylic aciduria plus features of Refsum disease. These results highlight the role of key metabolic regulators, such as PPARa, in generation of the metabolic phenotype of inborn errors.

Program Nr: 159 from 2002 ASHG Annual Meeting

**Transgenic models of LGMD1A: expression of altered human myotilin results in a dominant-negative phenotype.**

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Individuals affected with Limb-Girdle Muscular Dystrophy type 1A (LGMD1A) exhibit proximal leg and arm weakness, elevated serum creatine kinase levels, and a dysarthric pattern of speech. Muscle biopsies show reduced fiber size, fiber splitting, central nuclei, autophagic vesicles, and Z-line streaming. Positional cloning previously revealed a T57I missense mutation in the *myotilin* gene co-segregating with disease in the only known LGMD1A pedigree (Duke family 39). Myotilin protein is localized to the Z-line and we show here that myotilin antibodies stain Z-line streaming figures. We also report here a second LGMD1A family from Argentina, displaying a novel S55F *myotilin* mutation, and a strikingly similar clinical presentation.

To further elucidate the normal functions of myotilin protein, we are constructing a series of transgenic mice expressing modified human myotilin genes on a normal mouse background. One such line expresses wild-type human myotilin with an N-terminal c-myc epitope tag, under transcriptional control of the human skeletal actin promoter. These mice display strikingly abnormal, inwardly turned forelimbs, and walk on their elbows. Two transgenic mice have been evaluated with a full neurophysical and behavioral screen, including a grip strength assessment. Testing of additional mice is underway, but preliminary results show that forelimb grip strength is markedly reduced, and re-extension of the toes is delayed after the grip is broken. Coordination is normal in these mice, and the hindlimbs do not show any gross functional impairment. We hypothesize that the c-myc epitope tag is disrupting the known interactions with alpha-actinin or gamma-filamin, or is inhibiting some as yet unknown function associated with the N-terminus of myotilin protein.

Program Nr: 160 from 2002 ASHG Annual Meeting

**Overexpression of $p21^{Cip1}$ correlates with phenotypic severity and accelerated chondrocyte differentiation in FGFR3-related chondrodysplasias.** *L. Legeai-Mallet, C. Benoist-Lasselin, A. Munnich, J. Bonaventure.* INSERM U393, Department Genetics, Hopital Necker-Enfants Malades, Paris, France.

Achondroplasia (ACH) and thanatophoric dysplasia (TD) are human skeletal disorders of increasing severity accounted for by mutations in Fibroblast Growth Factor Receptor 3 (FGFR3). In an attempt to elucidate the disease causing mechanisms, several mouse models have been generated which save rise to variable and discordant phenotypes. Taking advantage of a large series of cartilage samples from ACH and TD fetuses, we investigated the consequences of FGFR3 mutations on the cartilage growth plates. Histological and immunohistochemical examination documented defective differentiation of hypertrophic cells which morphologically resembled pre-hypertrophic cells and strongly expressed Stat1, Stat5 and $p21^{Cip1}$ proteins. The extent of over-expression was directly related to the severity of the phenotype. Double staining procedures revealed overlap between FGFR3 and Stats/$p21^{Cip1}$ in the prehypertrophic/hypertrophic zone, thus indicating that constitutive activation of the receptor accounts for their over-expression. By contrast, expression of these proteins in the proliferative zone did not significantly differ from control cartilage. Likewise, primary cultured ACH and TD chondrocytes responded normally to the mitogenic effect of FGF9 and expression of collagen type X in the hypertrophic zone of cartilage was lower than in controls. Our results indicate that the proliferative capacities of chondrocytes from 15-30 weeks fetuses are not impaired by FGFR3 mutations whereas $p21^{Cip1}$ up-regulation accelerates their differentiation into pre-hypertrophic cells. We conclude that defective differentiation of prehypertrophic and hypertrophic chondrocytes is the main cause of longitudinal bone growth retardation in ACH and TD fetuses.

Program Nr: 161 from 2002 ASHG Annual Meeting

**Phenotypic consequences of gene dosage imbalance in chromosome engineered mouse models for del(17)p11.2 (Smith-Magenis syndrome) and it's reciprocal duplication.** *K. Walz[1], S. Carattini-Rivera[1], W. Bi[1], D. Mansouri[1], H. Vogel[4], R. Paylor[1], J.K. Lynch[3], J.L. Noebels[3], A. Bradley[2], J.L. Lupski[1].* 1) Dept Mol & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Sanger Center, Cambridge, UK; 3) Dept of Neurology,Baylor College of Medicine, Houston, TX; 4) Dept of Pathology, Stanford University Medical Center.

Contiguous gene syndromes (CGS) refer to a group of disorders associated with chromosomal rearrangements (deletions or duplications) in which the phenotype is thought to result from altered copy number of physically linked dosage sensitive genes. Smith-Magenis Syndrome (SMS) is a CGS associated with a deletion in (17)p11.2. The clinical phenotype includes craniofacial anomalies, heart and urinary tract abnormalities, seizures, behavioral difficulties, sleep abnormalities and mental retardation. The reciprocal duplication syndrome has been described for humans. The phenotype includes mild to borderline mental retardation and behavioral difficulties.Human chromosome 17p11.2 is syntenic to the 32-34 cM region of murine chromosome 11. By chromosomal engineering technology we created rearranged chromosomes carrying the deletion (Df17) or duplication (Dp17) of a region on mouse chromosome 11 that spans the genomic interval commonly deleted in SMS patients.Heterozygous Df17, Dp17 or Df17/Dp17 were analyzed in a C57BL/6-Tyr c-Brd- 129S5/SvEvBrd mixed background in order to assess phenotypic consequences of gene dosage imbalance in this region. Homozygous animals were found for Dp17, but not for Df17. Analysis of heterozygous mice indicated haploinsufficiency phenotypes. Craniofacial abnormalities were observed in Df17 mice with virtually complete penetrance, whereas 20% of these mice presented seizures. No craniofacial abnormalities nor seizures or abnormal EEG were found in any of the other genotypes analyzed.Df17 animals are overweight compared to their wt littermates while the Dp17 animals are underweight. Male reduced fertility was also observed in Df17 animals. Our murine models represent a powerful tool to analyze the consequences of gene dosage imbalance in this region and investigate the molecular genetic basis of both SMS and dup(17)p11.2.

Program Nr: 162 from 2002 ASHG Annual Meeting

**Intra-lysosomal cystine accumulation in mice lacking cystinosin, the protein defective in cystinosis.** *S. Cherqui[1], C. Sevin[1], G. Hamard[2], V. Kalatzis[1], M. Pequignot[3], K. Cogat[3], M. Abitbol[3], M. Broyer[1], M.C. Gubler[1], C. Antignac[1,4].* 1) Inserm U423, Necker Hospital, Paris, France; 2) Plateau de Recombinaison Homologue, Institut Cochin, Paris, France; 3) CERTO, EA2502, Paris 5 University, Paris, France; 4) Department of Genetics, Necker Hospital, Paris, France.

Cystinosis is an autosomal recessive disorder characterized by an accumulation of intra-lysosomal cystine. The causative gene, *CTNS*, encodes cystinosin, a 7 transmembrane domain protein, which we recently showed to be the lysosomal cystine transporter. We cloned and characterized the murine homologue of *CTNS*, *Ctns*, and the encoded amino acid sequence is 92.6 % similar to cystinosin. We report the first *Ctns* knockout mouse model generated using the promoter trap approach. We replaced the last four *Ctns* exons by a IRES-bgal-neo cassette and showed that the truncated protein was mis-localized and non-functional. *Ctns-/-* mice accumulate cystine in all organs tested, and cystine crystals, pathognomonic of cystinosis, were observed. *Ctns-/-* mice developed ocular changes similar to those observed in affected individuals, bone and muscular defects and behavioral anomalies. Interestingly, *Ctns-/-*mice did not develop signs of a proximal tubulopathy, or renal failure. The comparison of the two species may bring to light the cause of this pathology seen in children with cystinosis, the exact origin of which remains a major question. A preliminary therapeutic trial using an oral administration of cysteamine was carried out and demonstrated the efficiency of this treatment for cystine clearance in *Ctns-/-* mice. This animal model will prove an invaluable and unique tool for testing emerging therapeutics for cystinosis.

Program Nr: 163 from 2002 ASHG Annual Meeting

**Mutations in the CBP PHD finger disrupt Histone Acetyl Transferase (HAT) activity and cause RTS.** *M.H. Breuning[1], J.H. Roelfsema[1], E. Kalkhoven[1], H. Teunissen[1], Y. Ariyurek[1], R.C.M. Hennekam[2], D.J.M. Peters[1]*. 1) Dept Clinical Genetics, Leiden Univ, Leiden, Netherlands; 2) Dept of Clinical Genetics, Amsterdam Medical Center.

Rubinstein-Taybi Syndrome (RTS) is well-defined by a characteristic face, broad thumbs, broad big toes and mental retardation as major clinical hallmarks. RTS is caused by mutations, mostly de novo, in the gene coding for CREB Binding Protein (CBP). These mutations range from deletions removing the entire gene, to point mutations. CBP is a protein involved in many signal transduction pathways. It forms a physical bridge between various transcription factors and the basal transcriptional machinery. In addition, it is a potent histone acetyltransferase. Acetylation of histones is a mechanism involved in the regulation of gene expression. Transcriptionally active chromosomal loci frequently have acetylated core histones. Mutation analysis on the HAT domain of CBP was performed, using DGGE and SSCP. We identified 8 mutations in 39 patients. Two of these mutations affect the PHD finger, at the N-terminal end of the HAT domain. One is a missense mutation at amino acid 1278 and the other is a mutation at the splice acceptor site of intron 21. This mutation results in an aberrantly spliced mRNA without exon 22, causing an in frame deletion. The effect of both mutations on the HAT activity of CBP was investigated with HAT assays. Expression constructs were used to generate CBP with the same mutation as found in the patients. In contrast to wild-type CBP the mutated forms of the protein were not able to acetylate histones at all. In addition, the effect of the mutations on gene expression was investigated with luciferase reporter constructs. Indeed, loss of HAT function results in almost complete reduction of luciferase expression. The mutations in the RTS patients and the subsequent biochemical analysis using HAT assays and luciferase reporter assays, provide in vivo and in vitro evidence that the PHD finger is functionally part of the HAT domain of CBP. Furthermore, these mutations indicate that loss of the histone acetyltransferase function in CBP results in RTS.

Program Nr: 164 from 2002 ASHG Annual Meeting

**Simple mutations in the coding region of three acyl-CoA dehydrogenase genes may lead to skipping of the constitutive exon due to disruption of potential splice enhancer sequences or mRNA secondary structures.** *B.S. Andresen[1,2], K.B. Nielsen[1,2], P.P. Madsen[1,2], T.J. Corydon[1], J. Kjems[3], A.R. Krainer[4], O.N. Elpeleg[5], L.D. Schroeder[1,2], N. Gregersen[2]*. 1) Inst Human Genetics, Aarhus Univ, Aarhus, Denmark; 2) Research Unit for Molecular Medicine, Aarhus University Hospital; 3) Institute of Molecular and Structural Biology, Aarhus University, Denmark; 4) Cold Spring Harbor Laboratory, New York, USA; 5) Share-Zedek Metabolic Center, Jersualem, Israel.

In recent years examples from several genes where point mutations in the coding region lead to missplicing without directly involving splice consensus sequences have been reported. Disruption of exon splice enhancer (ESE) sequences and/or mRNA secondary structure have been used as explanations. We have found that this phenomenon exists in all of three investigated acyl-CoA dehydrogenase (MCAD, VLCAD and SBCAD) genes, and studied the mechanisms by investigation of selected disease-causing mutations using transfection of minigenes in CHANG-cells. Studies with our MCAD minigene showed the following: Optimization of the suboptimal splice consensus sequences that flank the skipped exon abolished the mutation-induced exon skipping by alleviating the need for an ESE. This MCAD ESE could be predicted by computer analysis and had a high score for the splicing factors SF2/ASF and SRp40. The correlation between the calculated scores for mutant forms of the ESE and the level of missplicing was investigated. Missplicing of the mutant MCAD minigene as well as that from the endogenous MCAD gene in patient cells could be corrected by cotransfection of SF2/ASF, but not by other splicing factors. Similar studies are underway with a SBCAD missense mutation that may cause ESE inactivation. Studies of a VLCAD missense mutation using our VLCAD minigene suggests that disruption of mRNA secondary structure also plays a role in exon skipping. We conclude that it is not uncommon that simple coding region mutations lead to exon skipping either as a result of disruption of ESE's or mRNA secondary structures, which are necessary for correct splicing of exons located in a suboptimal context for splicing.

Program Nr: 165 from 2002 ASHG Annual Meeting

**Heterologous expression reveals differential cellular trafficking of membrane-associated proteins.** *J.E. Mickle, C. Blaschak, C. Yurk, G. Cutting.* Inst Genet Med, John Hopkins Univ Sch Med, Baltimore, MD.

Improper cellular trafficking of membrane-associated proteins underlies numerous genetic diseases; cystic fibrosis (CF) is a prime example. The most common CF mutation, L F508, alters the folding of CFTR such that the mutant protein no longer reaches its targeted location, the apical membrane, where it would otherwise mediate chloride secretion across epithelia. Many CF therapies aim to shuttle the LF508 mutant protein to the apical membrane so that function can be restored. But, the mechanisms that facilitate CFTR trafficking to this location are not clear. To elucidate these mechanisms we employed a comparative approach. CFTR has been identified in numerous species; the most divergent form is from killifish. Since killifish (kf)CFTR also localizes to the apical membrane of native epithelia, we hypothesized that the trafficking mechanism is evolutionarily conserved. Thus, we expressed kfCFTR in polarized mammalian epithelial cells and assessed protein localization. Firstly, the cDNA of green fluorescent protein (GFP) was fused to the amino terminus of full-length kfCFTR cDNA via a successive subcloning strategy to distinguish kfCFTR expression from any trace of endogenous CFTR upon heterologous expression in mammalian epithelial cells. Then, the full-length GFP-kfCFTR cDNA construct was transiently expressed in MDCK II cells using standard transfection protocols. Cells were cultured for three days at $37^{o}$C and 5% $CO_2$ with supplemented media. Then the cells were fixed, permeabilized, and stained with dyes for F-actin (rhodamine phalloidin, Molecular Probes) and nuclei (DAPI, Sigma). Accordingly, fluorescent images were captured with a Zeiss LSM 410 confocal microscope. Results show that GFP alone is expressed throughout the cytoplasm, but GFP-kfCFTR colocalizes with F-actin to the plasma membrane, particularly at the apical and lateral surfaces. In contrast, GFP-human CFTR localization is principally apical in MDCK cells (J Clin Invest 104:1353-1361, 1999). This suggests that kfCFTR can interact sufficiently with mammalian orthologs for membrane localization, but that specific apical localization is precluded by differences in kfCFTR sequence.

Program Nr: 166 from 2002 ASHG Annual Meeting

**Preliminary validation of genomic microarrays for routine use in prenatal screening for chromosomal imbalances.** *M.S Mohammed[1], J. Kang[2], N. Dzidic[2], R. Locker[2], E. Vilain[3], C. Bacino[1], W.W. Cai[1], R. Naeem[1]*. 1) Spectral Genomics, Inc, Houston, TX; 2) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Dept. of Human Genetics and Pediatrics, UCLA, CA.

Screening is defined as,The identification among apparently healthy individuals,of those who are sufficiently at risk of a specific disorder to justify a subsequent diagnostic test or procedure, or in certain circumstances, direct preventive action.(Nicholas J.Wald). FISH-methodologies such as AneuVisionTM have helped our approach to prenatal screening for five chromosome aneuploidies However; the use of locus-specific FISH probes to a limited subset of chromosomes precludes the ability to detect segmental imbalances of these chromosomes and other aneuploidies, which represent a significant portion of prenatal chromosome abnormalities. With the aim of developing a genome-wide prenatal screening for chromosomal imbalances we developed a high-density BAC/PAC genomic array. In addition, we have developed protocols with which DNA samples less than 100ng can be analyzed in less than 48 hours. A retrospective study was performed on discarded samples representing a spectrum of chromosome abnormalities, including segmental monosomies, trisomies, double segmental imbalances,complex marker chromosomes and examples of chromosome mosaicism. Test and reference genomic DNAs were differentially labeled with fluorochromes on Day 1 and hybridization was performed overnight. On Day 2, the BAC arrays were scanned and bioinformatically analyzed. Analysis of all samples was performed in a blinded fashion. Cyto,FISH and BAC CGH data from unbalanced aberrations for which clone coverage was available on the array showed concordance. Data from cytogenetically characterized mosaicism including X chromosome showed consistent ratio divergence. Since this technology is in principle not limited to number of BAC clones but rather to genome sequence mapping data,it is clear that more complete sequencing data and progressive innovations in DNA preparation,labeling and hybridization,will introduce genome microarrays as a rapid,reliable and comprehensive prenatal screen for all chromosomal aneuploidies and segmental imbalances.

Program Nr: 167 from 2002 ASHG Annual Meeting

**Origin of Chaotic Embryos in Preimplantation Development.** *A. Kuliev, J. Cieslak, Y. Verlinsky.* Reproductive Genetics Inst Inc, Chicago, IL.

The nature and origin of chaotic embryos, as well as high rate of mosaicism in preimplantation development are not known. Although different mechanisms may be involved, the majority of these abnormalities may originate from female meiosis, contributing considerably to pregnancy failures in women of advanced maternal age. We studied 8362 oocytes obtained in 1297 IVF cycles from patients of advanced maternal age, using fluorescent in situ hybridization (FISH) analysis of the first (PB1) and second polar bodies (PB2), with application of commercial probes specific for five chromosomes (chromosomes 13,16, 18, 21 and 22) (Vysis). Of 6733oocytes with available FISH results, 3509 (52.1%) were with aneuploidies, comparable to the proportion of chromosomal abnormalities in cleaving embryos detected in preimplantation diagnosis for aneuploidies at the cleavage stage. Comparable involvement in aneuploidy was also observed for each chromosome studied, except for chromosome 16. Analysis of the types of abnormal oocytes showed that 45.1% of these abnormalities are of complex nature, involving the same chromosome in both meiotic divisions (21.5%), or different chromosomes (78.5%), of which 74.8% were with abnormalities of two, and 25.2% with abnormalities of three chromosomes studied. The complex abnormalities originated both in the first (30.1%) and second (22.1%) meiotic divisions. Based on these data, it may be concluded that half of embryos are chromosomally abnormal from the outset, with almost every second of them having complex aneuploidies,which may be due to chiasmata or spindle formation abnormalities. It may be suggested, that with further mitotic errors at the cleavage stage, which may represent the phenomenon of aneuploidy rescue, the embryos with complex abnormalities further result in mosaic or completely chaotic embryos and lost during preimplantation development or implantation.

Program Nr: 168 from 2002 ASHG Annual Meeting

**Single Blastomere Nucleus Conversion to Metaphase for Preimplantation Diagnosis of Translocations.** *Y. Verlinsky, J. Cieslak, S. Evsikov, V. Galat, A. Kuliev.* Dept Molec Genet, Reproductive Genet Inst, Chicago, IL.

Preimplantation genetic diagnosis (PGD) for chromosomal disorders by interphase FISH analysis has limitations for complete karyotyping. So single blastomere conversion to metaphase was developed to improve the accuracy of PGD for aneuploidies and translocations. To visualize single blastomere chromosomes, the blastomere nuclei were fused with enucleated or intact mouse zygotes, followed by fixing the resulting heterokaryons at the metaphase of the first cleavage division, or treating them with okadaic acid to induce premature chromosome condensation. Of 437 blastomeres exposed to this method, analyzable metaphases were obtained in 383 (88%), suggesting an acceptable efficiency, which in combination with FISH analysis allows a significant improvement in the testing accuracy of both maternally and paternally derived translocations. A total of 52 clinical cycles were performed using this technique, resulting in 38 transfers of balanced or normal embryos. Overall, PGD for translocations was applied in 94 clinical cycles, resulting in 66 transfers and 20 (30.3%) clinical pregnancies, from which only 5 spontaneously aborted (25% vs. 75% in the same couples prior to undertaking PGD), the others resulting in 13 healthy deliveries of 15 children (with the remaining two still ongoing). The follow up analysis of the embryos with unbalanced chromosomal rearrangements and of the pregnancy outcomes following the transfer of normal or balanced embryos, suggested the clinical acceptability of the PGD method applied, which resulted in more than a three-fold reduction of spontaneous abortion rate in translocation carriers.

Program Nr: 169 from 2002 ASHG Annual Meeting

**Further Characterization and Expansion of the Neurobehavioral Phenotype of The Child with Sex Chromosome Variations (SCV).** *C.A. Samango-Sprouse.* Dept Pediatrics, Children's Natl Med Ctr/GWU, Washington, DC.

Sex Chromosome Variations(SCV) occur one in 500 children and are largely undiagnosed. The learning profile of the school age child with SCV is characterized by a language based learning disablity with dyslexia, attentional deficits and auditory memory dysfunction. The neurodevelopmental profile of the young child with SCV has been described in the first year of life with neuromotor dysfunction,speech and language deficits (Samango-Sprouse, 2000, Samango-Sprouse and Law, 2001). These deficits have been characterized as an infantile presentation of developmental dyspraxia(Samango-Sprouse and Rogol, 2002). Since sensory dysfuntion is commonly observed in non-syndromic children with developmental dyspraxia, a prospective study of the sensory function in infants and children with SCV was undertaken. Sensory profiles (Dunn,1999) and comprehensive neurodevelopmental evaluations were completed on 36 children with SCV. The sensory profile assessed auditory, visual, vestibular, tactile, oral and multisensory processes. Children with SCV were found to be at risk with deficits in oral ($p<.05$) and vestibular($p<.05$)sensory processes when compared to population controls. The children with atypical vestibular and oral sensory processing had truncal hypotonia and speech delay. These sensory differences in oral and vestibular function further support the phenotype of motor planning deficits and infantile presentation of developmental dyspraxia (IDD). These sensory differences may explain the altered some of the behavioral responses these children have in both social and school settings. Appropriate oral sensation facilitates the acqusition of speech and language production. Vestibular function is an integral component of balance, motor planning and sequencing. These deficits in oral and vestibular processing as early as 12 months may predict learning disbilities and further investigation is underway. This study further expands the behavioral phenotype of the child with SCV. Genetic counseling of the famliy with a prenatally diagnosed fetus with SCV should discuss the need for comprehensive evaluation of all systems and increased incidence of sensory dysfunction and IDD.

Program Nr: 170 from 2002 ASHG Annual Meeting

**Congenital diaphragmatic hernia: associated defects and syndromes and their impact on mortality.** *M.M. Dott[1,2], L.Y. Wong[2], S.A. Rasmussen[2]*. 1) Epidemic Intelligence Service; 2) National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA.

Congenital diaphragmatic hernia (CDH), the displacement of abdominal contents into the thoracic cavity through a defect in the diaphragm, is a birth defect often associated with a poor outcome. Few large population-based studies of the condition have been completed and little is known about the risk factors for death. We ascertained infants born during 1968-1999 using the Metropolitan Atlanta Congenital Defects Program (MACDP), a population-based birth defects surveillance system, to assess defects and syndromes associated with CDH. In addition, we used MACDP, Georgia vital records, and the National Death Index to document vital status. Risk ratios and 95% confidence intervals (95% CIs) were calculated to assess the relationship between mortality associated with CDH and the presence of an additional major congenital anomaly or syndrome. We found 249 cases of CDH, 19 (8%) of which had an associated syndrome. Almost all of the associated syndromes were chromosomal abnormalities, most commonly trisomies. About one-third of patients without a diagnosed syndrome had an additional major congenital defect. The most commonly affected systems were the cardiovascular (16%), musculoskeletal (16%), and gastrointestinal systems (7%). Overall, survival to age 1 year improved from 19% (1968-1971) to 54% (1996-1999). However, among infants with both CDH and a syndrome, none survived to age 1 year. Thus, infants with CDH and a syndrome had a 1.8 (95% CI=1.6-2.1) times higher risk for death than infants with isolated CDH. Although not statistically significant, non-syndromic infants with at least one additional major congenital anomaly were also at higher risk of death than those infants with isolated CDH. Careful evaluation of infants with CDH, including genetics evaluation, is important because of the impact of additional defects and syndromes on prognosis.

Program Nr: 171 from 2002 ASHG Annual Meeting

**Malformation rates in offspring of mothers in the UK Epilepsy and Pregnancy Register: results from a 7 year prospective study 1996 2002.** *P. Morrison and on behalf of the UK epilepsy and pregnancy register.* Dept Medical Genetics, Belfast City Hosp, Belfast, Northern Ireland.

Objective:To determine relative risks for major and minor congenital malformations (MCM) of in-utero exposure to anti-epileptic drugs (AEDs). Design:Prospective, observational, registration and follow-up study. Subjects:Women with epilepsy who become pregnant, whether or not they are taking an AED, either singly or in combination, and who are referred before outcome of the pregnancy is known. Outcome measure: Malformation rate for each AED regime for exposed pregnancies. Results: Full outcome data are available on 2028 pregnancies from 1996 through May 2002, with 594 outcomes awaited. Monotherapy exposures account for 73% (MCM rate 4.3% [95% C.I. 3.4 5.6%]), polytherapy exposures for 21.5% (MCM rate 7.2% [95% C.I. 5.0 10.3%]), and no AED exposures for 5.5% (MCM rate 1.0% [95% C.I. 0.2 5.3%]) of cases. The crude MCM rate for carbamazepine (2.6% [95% C.I. 1.6 4.4%]) is significantly less than for sodium valproate (7.7% [95% C.I. 5.5 10.7%]). Of 293 known lamotrigine outcomes, the crude MCM rate (2.8% [95% C.I. 1.3 5.6%]) is less than for sodium valproate. Cardiac and gastrointestinal abnormalities were the main malformations noted in Lamotrigine pregnancies. Of 49 outcomes on Gabapentin, Ethosuxamide, Vigabatrin, Topiramate and Piracetam, 6% had major malformations Conclusions: The UK Epilepsy and Pregnancy Register is proving an effective method of collecting outcome data on large numbers of pregnancies occurring in women with epilepsy. The results suggest that there are differences in MCM rate, and type between AEDs. Newer drugs have a different profile compared to older medications, but monotherapy is significantly safer than polytherapy.

Program Nr: 172 from 2002 ASHG Annual Meeting

**The impact of an abnormal Ultrasound (US) on Cytogenetics: Experience from a large prenatal diagnosis center.**
*K.S. Reddy, B. Huang, M. Thangavelu.* Director, Genzyme Genetics, Orange, CA.

Sophisticated ultrasound equipment is refining the sonographic identification of fetal abnormalities and serves as a powerful aid in the detection of chromosome abnormalities. In this study we track the efficacy of ultrasound markers on cytogenetics. Of 79652 prenatal samples karyotyped, 2416 [3.0%] were abnormal. An abnormal US finding was an indication in 4594 [5.8%] cases and 739 [16.2%] had an abnormal karyotype. A majority of US abnormalities were detected in the second (82.3%) and third (14.1%) trimester. An abnormal karyotype was seen in 10% of fetuses with a single US abnormality and 36.47% of fetuses with ³2 US abnormalities. The chromosome abnormalities in cases with a single US abnormality were: +21 [30.3%], +13 [10.2%], +18 [25.4%], XO[15.2%], sex chromosome [0.8%], other aneuploidy [1.4%], triploid [4.7%], mar [1.2%], structural [10%], and microdeletion (0.6%). An ultrasound abnormality was found in 90.2% of cases with a 45,X karyotype, 22.8% with +21, 65% with +13 and 60.3% with +18. Some structural abnormalities were subtle such as deletions of 1pter, 8pter and rearrangements of 18p/q, 4p and 5p. A total of 118 fetuses with cardiac defects were tested by FISH for DiGeorge syndrome and 4 (3.4%) were positive. The percentage of chromosome abnormalities detected in malformations affecting the head was 4.5%, neck-nuchal translucency was 20.1%, heart was 8.6%, GI was 8.2%, skeleton & limb defects was 10.5%, and hydrops & oligohydramnios was 33.9%. The details will be presented. Women with an US abnormality who were <35 years had 13.3% while >35 had 25% chromosome abnormality. In conclusion, ultrasound examination increases the detection rate of chromosome abnormality (from 2.2 % to 16.2%). Multiple (³2) sonographic anomalies increases the risk for an aberrant karyotype to 36.4%. Mothers >35 years of age and US abnormality have a high risk of 25% for a chromosome abnormality. Furthermore, association of ultrasound abnormalities with chromosome abnormalities presented in this study provides valuable information in helping patients to make decision regarding an invasive prenatal diagnosis procedure when a specific US abnormality is found.

Program Nr: 173 from 2002 ASHG Annual Meeting

**Guidelines for prenatal detection of the 22q11.2 deletion.** *D.M. McDonald-McGinn, D.A. Driscoll, S. Saitta, A. Jawad, M. Tonnesen, J.E. Ming, E. Goldmuntz, D. Canning, N. Spinner, B.S. Emanuel, E.H. Zackai.* Children's Hosp of Phila and University of Pennsylvania School of Medicine, Philadelphia, PA.

It is well-documented that congenital heart disease (CHD) is associated with the 22q11.2 deletion, and it has become routine to perform FISH analysis in fetuses with conotruncal cardiac anomalies. We have noted several findings in our 22q11.2 deletion population, identifiable during routine prenatal care, which could support deletion studies intrauterine. We found: renal problems in 31% of 80 patients (10% renal agenesis/multicystic dysplastic kidney and 5% hydronephrosis); polyhydramnios in 21/107 which is 20 fold > the general population incidence (GPI); pre- and post-axial polydactyly of the hands in 4/104 (4%) and post-axial polydactyly of the feet in 1/104 which are at least 10 fold > the GPI in both Caucasians and African Americans; and congenital diaphragmatic hernia in 3/370 which is also 20 fold > the GPI. Thus, these sonographic findings in conjunction with CHD should support fetal 22q11.2 deletion studies in order to provide appropriate prenatal counseling and management.

Furthermore, we identified 21 deleted parents ascertained because of an affected child, including a 20 year old mother with repaired tetralogy of Fallot (TOF) and LD. Subsequently we studied the parents of 30 consecutive patients prospectively and identified an affected mother with a history of a VSD and LD and a normal father with somatic mosaicism, yielding a 7% familial incidence of the deletion. We have also identified an additional family with either low level somatic mosaicism or germ line mosaicism. Thus, these findings suggest that parental FISH studies are now indicated in all families with an affected child to rule out somatic mosaicism and clinicians should caution non-deleted parents regarding the risk of germ line mosaicism. In addition, a history of CHD in a pregnant woman or her partner, especially in conjunction with a learning disability, even with no history of a previously affected child, should alert the clinician to consider parental 22q11.2 deletion studies for prenatal counseling.

Program Nr: 174 from 2002 ASHG Annual Meeting

**Use of cartilage cDNA microarrays to dissect the molecular pathways in thanatophoric and campomelic dysplasias.** *D. Krakow[1,2], E. Sebald[1,2], R. Pogue[2], D. Cohn[2,3]*. 1) Dept OB/GYN, Cedars-Sinai Medical Ctr and UCLA, Los Angeles, CA; 2) Dept of Ped, Cedars-Sinai Medical Ctr and UCLA, Los Angeles, CA; 3) Dept of Human Genetics, UCLA School of Medicine, Los Angeles, CA.

Thanatophoric dysplasia (TD) and campomelic dysplasia (CD) are prenatal-onset autosomal dominant skeletal dysplasias, both of which present with severe phenotypes. TD is due to activating mutations in FGFR3 and is a lethal in the perinatal period. CD results from haploinsufficiency for the SOX9 gene product. To determine the molecular pathways involved in these disorders, cartilage RNA was used to interrogate a 400-gene cartilage cDNA microarray. In TD, there was increased expression of the genes encoding SPROUTY2, calmodulin, and cartilage link protein. Interestingly, studies in Drosophila and the chick show that sprouty orthologues are both induced by and antagonize FGF signaling, constituting a negative regulatory feedback mechanism. Because of constitutive activation of FGFR3 in TD, increased SPROUTY expression was predictable, but whether SPROUTY can influence the activity of the mutant FGFR3 is not known. Decreased expression was seen for the COL10A1, IL8, MMP13 and vimentin genes. Decreased COL10A1 expression probably results from the lack of a normal hypertrophic zone in TD. In CD, decreased expression was seen for the BMP1, COL10A1, MMP1, MMP2, and MMP13 genes. BMP1 is a metalloproteinase which has multiple functions, including the cleavage of the C-terminal propeptide of procollagens I, II, and III, activation of lysyl oxidase, and processing of bigylcan. Mice deficient in Bmp1 showed abnormal extracellular matrices with thin collagen fibers and less overall matrix. Thus, decreased expression of BMP1 in campomelic dysplasia may contribute to the abnormal bone phenotype. Increased expression was demonstrated for TRA1 and RUNX3 and many heat shock protein genes, suggesting that SOX9 mutations affect a number of distinct regulatory pathways. These data demonstrate that analysis of gene expression on microarrays can identify altered gene expression in the osteochondrodysplasias, helping to elucidate the specific molecular determinants of phenotype in these diseases.

Program Nr: 175 from 2002 ASHG Annual Meeting

**Conception to obtain hematopoietic stem cells for transplant for Fanconi anemia: ethical and legal issues.** *A.D. Auerbach[1], J. Wagner[2], J. Kahn[2], S.D. Batish[1], M.R. Hughes[3]*. 1) Rockefeller Univ, New York, NY; 2) Univ. of Minnesota, Minneapolis, MN; 3) Wayne State Univ., Detroit, MI.

Patients affected with Fanconi anemia (FA) have progressive bone marrow failure and a predisposition to AML. Hematopoietic stem cell transplant (HSCT) is the only proven treatment for this; unrelated or mismatched related HSCT have been attempted in FA patients with poor outcome compared to matched sib HSCT. Eighty pregnancies were conceived from 1985-2002 with potential to provide a HSC donor for an FA patient. Only 13.75% of the total fetuses tested were suitable donors; the probability for this event to occur with each pregnancy is 18.75%. Preimplantation genetic diagnosis and in vitro fertilization (PGD/IVF) is a new option for families in this situation. This is now technically feasible, and has been performed for 10 IFAR families in groups FA-C and FA-A. The central ethical issues that arise from conceiving a child to be a HSC donor concern that child's rights and welfare; it is a central moral principal that we treat other persons as ends and never as mere means. The commitment of parents to nurture, care for and love the second child is the key factor in determining whether the conception is ethically acceptable. We have encountered 2 legal issues regarding parents seeking to pursue this option. (i) Malpractice suits have been brought on behalf of plaintiffs for injuries sustained because of delay in diagnosis of FA, leading to loss of opportunity to conceive a child to be a HSC donor for an FA sibling. (Susan Ferrell et al. v. Kenneth N. Rosenbaum, M.D. et al., No. 94-CV-1179 District of Columbia Court of Appeals; Sandra Dickson et al. v. St. Mary's Hospital et al, Docket # CV97-0138599, Superior Court of CT., District of Waterbury). (ii) While it is legal to use fetal HSC for transplant if a SPAB occurs, it is illegal to use these cells in the case of an induced abortion ("Prohibitions Regarding Human Fetal Tissue," PUBLIC LAW 103-43, Sec 112, 498B; JUNE 10, 1993). A recent FA pregnancy conceived to provide a HSC donor demonstrated that the definition of induced abortion can be a gray area. These issues have applicability beyond FA, and need further discussion.

Program Nr: 176 from 2002 ASHG Annual Meeting

**Uniparental isodisomy of chromosome 1q in a patient with Hutchinson-Gilford Progeria Syndrome.** *M. Eriksson[1], J. Singer[2], L. Scott[2], A. Dutra[1], M. Boehnke[2], F.S. Collins[1].* 1) National Human Genome Research Institute, National Institute of Health, Bethesda, MD; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI.

By molecular analysis of a patient with Hutchinson-Gilford Progeria syndrome (HGPS, OMIM#176670) we present evidence of an autosomal recessive gene for this disorder. HGPS is an extremely rare premature aging syndrome. Death occurs on average at age 16, usually from cardiovascular disease. The inheritance pattern of HGPS is not known. The presence of very few reported affected sibpairs and a modest paternal age effect, together with very few cases of known consanguinity, has led some to favor a sporadic dominant mechanism. However, a previous report of a consanguinous family with 4 affected siblings favors autosomal recessive inheritance. We have identified a patient with uniparental isodisomy of chromosome 1q. Microsatellite markers spaced on average every 2.5 cM on the q-arm of chromosome 1 (between markers D1S498 and D1S2836), showed complete homozygosity. The patient did not show any evidence of increased homozygosity for any other chromosomal region, indicating it is very unlikely this finding was due to inbreeding. SKY and G-banding (550 bands) on primary fibroblasts from the same individual showed a normal karyotype. In addition, results from a whole genome homozygosity mapping approach, comprising 18 HGPS patients and 20 unaffected first-degree relatives (from the Coriell Cell Repositories and the Progeria Research Foundation Cell Bank), showed increased homozygosity for a number of markers on chromosome 1q amongst those affected by progeria ($p<0.05$), though the evidence does not point neatly to a single small interval. Prior published examples of uniparental disomy for chromosome 1 have shown no evidence for imprinted loci on this chromosome. Thus, though we cannot rule out the coincidence of two rare genetic events, the association of complete homozygosity for chromosome 1q and HGPS is most consistent with an autosomal recessive inheritance pattern, wherein the isodisomic region of chromosome 1q in this patient carries an HGPS mutation.

Program Nr: 177 from 2002 ASHG Annual Meeting

**X-aneuploidy in women with trisomic spontaneous abortion.** *D. Warburton[1], A. Kinney`[2,3], K. Bocskay[1], J. Kline[1,3].* 1) Columbia Univ, New York, NY; 2) Research Foundation for Mental Hygiene, New York, NY; 3) NY Psychiatric Institute, New York, NY.

During a study of the relationship of ovarian aging to trisomy, we investigated the incidence of X-aneuploidy in lymphocyte cultures from women ascertained through a trisomic spontaneous abortion (n=47), compared to women with a spontaneous abortion with another chromosomal anomaly (n=21), a chromosomally normal spontaneous abortion (n=19) and a livebirth (n=56). Since X-aneuploidy increases with age, we argued it might be a measure of biological aging. Some previous studies found an increase in cells with X-aneuploidy in women with a trisomic pregnancy or recurrent spontaneous abortion. Others found no association. We scored for X-aneuploidy using FISH with an X-centromere-specific probe in 100 metaphase spreads and 400 interphase nuclei per case. Scoring was blind to case status. We used ordinary least squares regression analysis, adjusting for maternal age, to estimate associations between trisomy and the frequency of cells with 1X, 3+X and total X-aneuploidy. The mean frequency of 1X cells (3.8%) was about three times as high in metaphase spreads as in interphase nuclei (1.3%), suggesting that X chromosome loss occurred during in vitro cell divisions. Within all pregnancy outcome groups the frequency of cells with X -aneuploidy increased linearly with age. For the total sample, the frequency of 45,X cells in metaphases was 1.4% in women <25, rising to 5.1% in women over 40. The frequency of cells with additional X chromosomes was lower: 0.6% in women <25, rising to 1.9% in women over 40. In metaphases, the frequency of all X-aneuploidy rose an estimated 2.6% per 10 years of age. In interphases, X-aneuploidy rose an estimated 1.2% per 10 years of maternal age. X-aneuploidy rates did not differ between women with trisomic pregnancies and the three comparison groups.Our study confirms the well-known age-related increase in X-aneuploidy with a womans age, but found no association with history of a trisomic conception.

Program Nr: 178 from 2002 ASHG Annual Meeting

**Skewed XCI in women experiencing a pregnancy with meiotic nondisjunction.** *M.S. Penaherrera[1], C.L. Beever[1], R.H. Jiang[1], D.E. McFadden[2], M.R. Hayden[1], L.L. Field[1], D.K. Kalousek[2], C.J. Brown[1], W.P. Robinson[1].* 1) Dept Medical Genetics, U. British Columbia, Vancouver BC, Canada; 2) Dept Pathology,U. British Columbia, Vancouver BC, Canada.

Trisomy is estimated to occur in 6% of clinical pregnancies yet little is known of its etiology other than it is generally due to an error in maternal meiosis and the incidence increases with advanced maternal age. We previously reported a significant increase in the incidence of trisomic pregnancies in women with extremely skewed XCI and recurrent spontaneous abortions. To confirm this association, a group of 54 women were investigated who were ascertained on the basis of a prenatal diagnosis of trisomy mosaicism for which the trisomy was shown to be of maternal meiotic origin. Using methylation based assays for various X-linked genes (*AR, FMR1 and DXS6673E*), the XCI status of these cases was evaluated using DNA extracted from peripheral blood lymphocytes and compared to two sets of controls. The first control group consisted of 84 women with a chromosomally abnormal pregnancy that was not due to maternal meiotic nondisjunction. The second control group consisted of 93 females with no known associated genetic abnormalities. The results show a significantly higher level of extreme skewing (³90%) in mothers of trisomic pregnancies of meiotic origin (20%) than in either the first or second control population (6 %; p=0.01 and 8 %; p=0.02 respectively [Fishers exact test]). Maternal age was not a confounding variable in this analysis as assessed by logistic regression. Previous studies have shown that a reduced ovarian reserve is linked to increased risk of trisomic pregnancies. We hypothesize that the trisomic pregnancies in patients with skewed XCI are related to a reduction of follicular pool size by either of two mechanisms: X-linked mutation or reduction of embryonic precursor pool size. X-linked mutations may cause skewed XCI and accelerated follicular atresia. Alternatively, cell depletion of varied etiology during early embryonic development of these mothers could lead to extreme skewing as well as to a reduced number of ovarian follicles.

Program Nr: 179 from 2002 ASHG Annual Meeting

**Characterization of a t(X;2)(p11.2;q37) *de novo* associated with congenital anomalies: Positional identification of disrupted genes.** *N.T. Leach[1,2], S. Michaud[1,2], B.J. Quade[1,2], A.H. Ligon[1,2], A.M. Michelson[1,2,3], R.L. Maas[1,2], B.R. Korf[1,2,4], S.R. Herrick[1], H.L. Ferguson[1,4], C.C. Morton[1,2].* 1) Brigham and Women's Hospital, Boston, MA; 2) Pathology, Harvard Medical School, Boston, MA; 3) Howard Hughes Medical Institute; 4) Massachusetts General Hospital, Charlestown, MA.

A goal of the Developmental Genome Anatomy Project (DGAP) is to discover genes important in human development. We ascertained an individual in which developmental delay (delayed walking and speech difficulties), seizures, infantile hypotonia, obesity and livedo reticularis (a purplish network-patterned discoloration of the skin) was associated with an apparently balanced t(X;2)(p11.2;q37) *de novo*. We hypothesized that the chromosomal rearrangement is causative for the observed phenotype because some anomalies are among clinical features observed in individuals with 2q37 terminal deletions. Initial breakpoint mapping was done using FISH with BAC clones and further refinement was carried out by FISH with PCR products derived from breakpoint-spanning clones. The breakpoint on chromosome 2 is presumed by FISH mapping to disrupt diacylglycerol kinase delta (*DGKD*), separating the promotor and exon 1 from the remainder of the gene. Disruption of *DGKD* seems to be a plausible candidate for the proband's seizure phenotype in view of the recent finding that mice deficient in diacylglycerol kinase epsilon have altered seizure susceptibility (Rodriguez de Turco et al., 2001). Consistent with a potential role for *DGKD* in neural development or pathophysiology, whole mount *in situ* hybridization analyses of mouse and fly *DGKD* orthologs showed prominent expression in the prosencenphalon and mesencephalon of day 12 mouse embryos, and in the brain and ventral nerve cord of late stage *Drosophila* embryos. The reciprocal breakpoint on the X chromosome was mapped by FISH near to, and potentially within, *WDR13*, encoding a tryptophan-aspartic acid repeat-containing protein of unknown function. Further studies are underway to determine the potential roles of *DGKD* and *WDR13* in the individual's phenotype.

Program Nr: 180 from 2002 ASHG Annual Meeting

**Quantitative Fluorescent PCR for the diagnosis of the common aneuploidies: development, sensitivity and diagnostic relevance.** *N. Dunlop[1], P.K. Westwood[1], K. Mann[2], M.E. Porteous[1], J.P. Warner[1]*. 1) Molecular Genetics Service, Clinical Genetics Section, Edinburgh, Lothian, Scotland; 2) SE Thames Regional Genetics Centre, Guy's Hospital, London.

We present two multiplex quantitative fluorescent PCR (QF PCR) assays. Test 1: tetranucleotide markers (4) from each of chromosomes 13 18 & 21, PIC 0.69-0.93, size range 100 - 500 bp (D13S742, D13S634, D13S628, D13S305, D18S1002, D18S535, D18S391, D18S386, D21S11, D21S1411, D21S1437 & IFNAR). Test 2: 7 sex chromosome markers (size range 100-320bp), 5 X (DXS1189, HPRT, DXS981, DXS1187 & DXS6803, PIC 0.75 - 0.87) 1 XY homologous (X22, PIC 0.87) and 1 X/Y specific (amelogenin). Test 1 is used to screen all antenatal and some pediatric referrals, test 2 supplements test 1 only when scan findings or clinical picture are suggestive of X or Y aneuploidy. We have evaluated the ABI 377 and ABI 3100 sequencers. The ABI 3100 sequencer is more rapid, reliable and sensitive. Electropherograms are analysed with Genescan™ and Genotyper™ software and data exported to Excel™. Allele area ratios are calculated. Statistical analysis of peak ratios for individual markers yield very tight distributions. Pure triploidies are unequivocally identified with no false positives (n>1000). Admixture experiments using various DNAs including a parthenogenetic cell line (haploid maternal only) have determined QF-PCR's sensitivity. Mosaicism of 10-14% is detected for extra alleles and 15- 20% when allele ratios are used. Currently all specimens are karyotyped (G banding) and high-risk cases analysed by interphase FISH. We present three cases showing strengths and weaknesses of QF-PCR. QF PCR has proved as sensitive as interphase FISH. G banding can reveal important structural insights but we have seen that selection under cell culture may alter mosaicism ratios. We recommend adoption of QF PCR as part of the standard molecular cytogenetic repertoire. It is highly sensitive, uses directly prepared DNA, has a low cost per test, low operator time requirement and routinely gives reporting times of 24-48 hours. In particular this test gives rapid reassurance for amniotic fluid testing where maternal age is the only risk factor.

Program Nr: 181 from 2002 ASHG Annual Meeting

**Further evidence of a tumor suppressor gene at 7q22 in malignant myeloid diseases.** *C.G. von Kap-herr, M.J. Pettenati, V. Shashi.* Pediatrics/Medical Genetics, Wake Forest Univ Sch Med, Winston-Salem, NC.

Monosomy of chromosome 7 or partial deletion of 7q is seen in 10% of Myelodysplastic syndromes (MDS) and Acute Myeloid Leukemia (AML), in as many as 50% of therapy related MDS/AML, and is associated with a poor prognosis. Studies using FISH have reported 7q22, 7q31, 7q33 and 7q35-36 as commonly deleted regions. It is believed that a tumor suppressor gene(s) exists within this region(s).

To further characterize the deletion region(s) in 7q22, we hybridized 30 overlapping YACs spanning 7q11.23-q36 and a 7q subtelomeric probe to 23 patients with MDS who had a cytogenetic deletion/translocation of 7q. Terminal (12/23) and interstitial (11/23) deletions occurred with equal frequency. In 11/23 cases, the breakpoint was through 7q22. In the majority (7/11) of the patients with a 7q22 breakpoint, the break occurred in or near YAC887C2, thus establishing this region as a common breakpoint. In two patients with 7q22 abnormalities [t(7;9) and add(7)], split signals were seen within two overlapping YACs (942E8 and 887D10, respectively) located just distal to the common breakpoint region defined by YAC 887C2. A putative tumor suppressor gene CUTL1, located within both 942E8 and 887D10, has been previously reported deleted in an MDS patient with an apparent balanced translocation of 7q22.

Our results indicate that the common breakpoint region in 7q22 is at or near YAC887C2, thus establishing this region as a potential site of a tumor suppressor gene(s). Additionally, our 2 patients with a perturbation within this region add strength to the possibility that CUTL1 may be a tumor suppressor gene and may play a significant role in the pathogenesis of MDS.

Program Nr: 182 from 2002 ASHG Annual Meeting

**Understanding the Molecular Mechanism of Dysregulated *HMGA2* Expression in Uterine Leiomyomata with 12q15 Rearrangements.** *D.M. Neskey[1], P. Dal Cin[2,3], S. Weremowicz[2,3], B.J. Quade[2,3], C.C. Morton[1,2,3]*. 1) Depts of Obstetrics, Gynecology, and Reproductive Biology; 2) Pathology, Brigham & Women's Hospital; 3) Harvard Medical School, Boston, MA.

Rearrangements in 12q15 are frequently encountered in cytogenetic studies of uterine leiomyomata (UL), and have been mapped to the *HMGA2* locus. In an effort to understand the role of *HMGA2* in the biology of these benign tumors, we performed FISH analyses using a variety of different 5' and 3' cosmids in the *HMGA2* locus to ascertain precisely the chromosomal breakpoints in a series of 38 UL with 12q15 rearrangements. In tumors with a breakpoint 5' of *HMGA2* (n=22), 20 cases involved a der(14)t(12;14)(q15;q24) and two tumors had rearrangements of 12q15 with chromosomal partners other than 14. Eight UL had breakpoints 3' of *HMGA2* of which five involved the 14q24 region and three involved other chromosomal regions. Nine cases involved both 5' and 3' breakpoints. In sum, the majority of t(12;14) breakpoints occur 5' of *HMGA2* (n=20/25), while the majority of rearrangements involving 12q15 and a partner chromosome other than 14 were 3' of *HMGA2* (n=3/5). Furthermore, at least 29/38 UL had breakpoints outside of the coding region. Of the nine tumors with both 5' and 3' rearrangements, six were unclassified insertions and three had 3' breakpoints that could be either in or beyond the coding region. Thus, all breakpoints may leave the entire *HMGA2* coding region intact. Following reports of *RAD51L1* as the translocation partner gene from 14q23-24, we assessed by RT-PCR a subset of these UL, some with 5' and 3' rearrangements, for *HMGA2*, *RAD51L1*, and their possible fusion transcripts. All tumors tested (n=10) were found to express *HMGA2* and *RAD51L1*. In 4/10 UL, two with 3' and two with 5' rearrangements, *HMGA2-RAD51L1* transcripts were detected. Because the *HMGA2-RAD51L1* fusions were not observed in all cases, and because *HMGA2* transcripts were present in all tumors with t(12;14), it remains to be known whether the *HMGA2-RAD51L1* fusion transcript is a critical biological event in UL or whether dysregulated *HMGA2* expression in itself is the important molecular mechanism.

Program Nr: 183 from 2002 ASHG Annual Meeting

**Familial versus sporadic ovarian tumors derived from Israeli-Ashkenazi women: characteristic genomic alterations analyzed by metaphase CGH and genomic microarray-based CGH.** *O. Israeli[1], S. Rienstein[1], E. Freidman[2], G. Barkai[1], B. Goldman[1], L. Peleg[1], G. Ben Baruch[3], W.H. Gotlieb[3], A. Aviram[1].* 1) Human Genetics, Sheba Medical Center, Israel; 2) Oncogenetics Unit, Sheba Medical Center, Israel; 3) Gynecology-oncology Department, Sheba Medical Center, Israel.

Worldwide, 5%-10% of ovarian carcinomas are familial, as a result of an inherited gene or genes, mainly BRCA1 and BRCA2. In the Jewish population, the rate of the familial ovarian carcinoma is higher and is associated with frequent specific mutations in the BRCA1 and BRCA2 genes. In order to get an overview of the genetic events leading to the development of familial and sporadic ovarian tumors and to identify chromosomal regions that may contain genes important in tumor progression, we employed the Comparative Genomic Hybridization (CGH) technique.A total of 49 invasive epithelial tumors: 29 sporadic tumors, 11 from a BRCA1 mutation (185delAG) carriers and 9 BRCA2 mutation (6174delT) carriers were analyzed. The average number of genetic alterations was significantly (alpha=0.085) higher in familial tumors (8.8 per tumor in BRCA1 and 7.4 in BRCA2) compared to the sporadic group (4.3 per tumor). The pattern of the chromosome amplifications resembles in the 3 groups and the most common amplifications detected were chromosomes 8q, 3q and 2q. The pattern of chromosome deletions varied between the groups. Among the BRCA1 group the most common deletions were in chromosomes 9 and 19 and in chromosomes 1p, 4q, 16 and 22. The BRCA2 group showed a lower frequency of deletions. Deletion of chromosome 16 was the most frequent one. No specific chromosomal deletion was significantly indicated in the sporadic group. In subsequent analysis we combined conventional CGH and genomic microarray technologies. Tumors were selected for analysis of gene amplification by genomic microarray-based CGH (Vysis), in order to search for gene amplifications associated with the development and progression of ovarian cancer. Our results demonstrated that 1. Familial ovarian tumors exhibit a significantly higher number of chromosomal aberrations and genomic imbalances. 2. Nonrandom genetic changes were characterized in the BRCA1 and BRCA2 groups.

Program Nr: 184 from 2002 ASHG Annual Meeting

**Human Rad51 protects cells in S/G2 phase from radiation-induced chromosome rearrangements.** *T. Haaf[1], E. Raderschall[2], I. Grandy[2], E. Fritz[3]*. 1) Institute of Human Genetics, Mainz University School of Medicine, Mainz, Germany; 2) Max Planck Institute of Molecular Genetics, Berlin, Germany; 3) Institute of Radiation Biology, GSF, Munich.

In order to assess the effects of Rad51-mediated recombinational repair on chromosome stability, both chromosome-type and chromatid-type aberrations were scored in constitutively Rad51-overexpressing rat fibroblasts and parental controls following treatment with different doses of gamma irradiation. The number of chromosome breaks (gaps, deletions and rings) was the same in the two populations. However, the number of chromatid breaks (gaps and fragments) and exchanges (triradials), which result from double-strand breaks (DSBs) generated during or after replication, was significantly reduced in Rad51-overexpressiong cells.

In conceptually related experiments, synchronized chick embryo fibroblasts (CEF) were transiently transfected with human Rad51 gene activity or control vector. The frequency of radiation-induced translocations between macrochromosomes was measured by spectral karyotype analysis. After irradiation of S or G2 phase cells with a dose of 6 Gy, the macrochromosome translocation frequency was significantly lower in the Rad51-transfected cultures, compared to controls. However, the transfected Rad51 had no protective effect in cells that were irradiated during G1 phase. Collectively, our experiments suggest an increased recombination and repair function of human Rad51 in S/G2 phase. Homologous recombination plays an important role in DSB repair in replicating mammalian cells.

In CEF cells the microchromosomes are usually distributed throughout the entire nucleus and, thus, in close physical contact with the macochromosomes, whereas in DT40 cells the microchromosomes are sequestered in the center of the nucleus. The frequency of radiation-induced translocations between microchromosomes and macrochromosomes was significantly higher in CEF than in DT40. Evidently, higher-order nuclear organization provides the structural basis for the formation of cell-type specific chromosome rearrangements.

Program Nr: 185 from 2002 ASHG Annual Meeting

**Correlating array CGH with gene expression and sensitivity to drugs in a panel of 60 human cancer cell lines.**

*K.J. Bussey[1], K. Chin[2], W.C. Reinhold[1], S. Lababidi[1], F. Gwadry[3], U. Scherf[3], Ajay[4], G. Tonon[5], A. Roschke[5], K. Stover[5], I. Kirsch[5], D.A. Scudiero[6], J.W. Gray[2], J.N. Weinstein[1]*. 1) Laboratory of Molecular Pharmacology, National Cancer Institute, Bethesda, MD; 2) University of California San Francisco Cancer Institute, San Francisco, CA; 3) GeneLogic, Gaithersberg, MD; 4) Celera Genomics, Rockville, MD; 5) Laboratory of Genetics, National Cancer Institute, Bethesda, MD; 6) SAIC-NCI-Frederick Cancer Research and Development Center, Frederick, MD.

We have used the NCI60 cell line panel as a model system to study the relationship of DNA copy number to mRNA expression level and drug sensitivity. Our analysis focused on 64 of 353 genes assayed by array CGH (CGHa). These genes had mRNA expression levels assessed by cDNA microarray and corroborated by data from oligonucleotide chips. The average Pearson correlations of CGHa with gene expression were 0.29 (SEM +/- 0.026) and 0.27 (SEM +/- 0.027) for the cDNA and oligonucleotide comparisons, respectively. There were no instances of statistically significant negative correlations. Approximately 2% of all possible comparisons among the 64 genes yielded statistically significant correlations between the DNA copy number of one gene and the expression level of a different gene. Most of the relationships seen have not been reported previously, but a few, such as the association of ERBB2 over-expression with 3p LOH in some cancers, were supported by evidence in the literature. We also examined the correlation between DNA copy number and drug sensitivity for 118 drugs with putatively known mechanisms of action. This examination identified the known inverse relationship between ABCB1 (i.e. MDR1) amplification and sensitivity to substrates of its gene product, P-glycoprotein, as well as a negative correlation between sensitivity to the enzyme L-asparaginase and DNA copy number of genes flanking asparagine synthetase. L-asparaginase has been used since the early 1970's to treat some cases of ALL, and our studies have suggested asparagine synthetase as a molecular marker to identify subgroups of patients with other cancer types who would also benefit from treatment with the enzyme.

Program Nr: 186 from 2002 ASHG Annual Meeting

**Mesenchymal stem cell transplants in acid sphingomyelinase deficient mice improves their neurological disease and extends their lifespan.** *H. Jin, X. He, E.H. Schuchman.* Human Genetics, Mount Sinai Sch Medicine, New York, NY.

Types A and B Niemann-Pick disease (NPD) result from deficient acid sphingomyelinase (ASM) activity. A mouse model of NPD (ASMKO mice) has been constructed that develops progressive lipid accumulation in various tissues, ataxia associated with Purkinje cell neuron dropout, and death by about 6-8 months of age. Previous studies in this mouse model have demonstrated that residual ASM activities as low as ~1-2% of normal in the brain could significantly alter the progression of CNS disease, and that activities of ~8% of normal could completely prevent it. To evaluate the potential of bone marrow-derived mesenchymal stem cells (MSC) to engraft and treat the CNS disease in ASMKO mice, we retrovirally-transduced MSCs from normal mice to overexpress ASM, and then transplanted ~10E5 transduced cells into the hippocampus and cerebellum of young ASMKO animals. Following transplantation, about half of the treated mice had improved rotarod performance compared to untreated mice, and survived up to at least 10 months. Histological analysis of the CNS at various times post-transplant revealed the positive effects of this gene therapy procedure on lipid storage and Purkinje cell numbers. We next combined intracerebral MSC transplants with BMT, since it had been previously shown that BMT alone led to partial CNS improvements. Indeed, the two procedures were synergistic and led to dramatic CNS improvements in the treated animals. However, an immunologic response occurred against the expressed human enzyme, limiting the duration of the experiment. We conclude that bone marrow-derived MSC may be very useful for cell-based therapies of Type A NPD and other neurodegenerative lysosomal storage disorders, particularly because this same cell type may be used to treat both the visceral organ and CNS complications associated with these diseases.

Program Nr: 187 from 2002 ASHG Annual Meeting

**Enzyme therapy for lysosomal acid lipase deficiency in the mouse model.** *H. Du[1], M. Levine[2], D. Witte[3], G. Grabowski[1]*. 1) Dept Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Genzyme Corporation, Boston, MA; 3) Division of Pathology, Children's Hosp Medical Ctr, Cincinnati, OH.

Lysosomal acid lipase (LAL) is a critical enzyme for the hydrolysis of the triglycerides (TG) and cholesteryl esters (CE) in lysosomes. Its deficiency produces two human phenotypes: Wolman disease (WD) and cholesteryl ester storage disease (CESD). An LAL null (lal-/-) mouse model was created and closely mimics the human WD/CESD. To test the potential for enzyme therapy and cellular targeting, recombinant human LAL expressed in Pichia pastoris (phLAL) or a CHO cell line (chLAL) were purified, and administrated to lal-/- mice by intra peritoneal (i.p.) injections. Following i.p. injection (240 min), cellular uptake of phLAL and chLAL was detected in Kupffer cells and splenic macrophages in lal-/- mice. In addition, LAL was detected in some hepatocytes with chLAL, but not with phLAL injected mice. The total recovery of injected enzyme in the liver was 27% for phLAL and 75% for chLAL. In lal-/- mice receiving 10 injections (every third day) of either phLAL or chLAL, both enzymes had changed the yellow hepatic color to normal and reduced the liver weight by 50-58%. Histological analyses showed that both enzymes reduced lipid storage in Kupffer cells, in splenic macrophages, and in small intestine. However, chLAL also eliminated lipid storage in hepatocytes while phLAL did not. Neither enzyme corrected the lipid storage in adrenal glands or lymph nodes. These studies provide "proof of principle" for phLAL enzyme therapy in human WD and CESD and specific cellular targeting and metabolic effects of enzymes with differential carbohydrate modification.

Program Nr: 188 from 2002 ASHG Annual Meeting

**Mouse models of X-Linked adrenoleukodystrophy and phenotypic correction by transgenic overexpression of the ALDR gene: functional redundancy at the peroxisomal membrane? Aurora Pujol, Carme Camps, Colette Hindelang, Elisabeth Metzger, Marisa Giros, Teresa Pampols* and Mandel JL.IGBMC, Strasbourg, France and *Institut de Bioquimica Clinica, Barcelona, Spain.** *A. Pujol[1], C. Camps[1,2], C. Hindelang[1], E. Metzger[1], M. Giros[2], T. Pampols[2], J.L. Mandel[1]*. 1) Human Molecular Genetics, IGBMC, Strasbourg, France; 2) Institut de Bioquimica Clinica, Barcelona, Spain.

X-linked adrenoleukodystrophy is a severe neurological disorder presenting with central or peripheral demyelination and impaired function of adrenals. X-ALD patients accumulate very long chain fatty acids (VLCFA) in plasma and tissues, notably in the adrenal cortex and nervous system. This disease is characterised by extensive phenotypic variability, even among patients sharing the same mutation. The two main neurological phenotypes are the severe childhood cerebral form and the slowly progressive adult adrenomyeloneuropathy. A mouse model of the disease also accumulates VLCFAs in target organs, and has recently been shown to develop an adrenomyeloneuropathy-like phenotype (Pujol et al, Hum Mol Genet 2002). The gene mutated in the disease (ABCD1) codes for a peroxisomal ABC transporter protein (ALDP). Its closest homologue (ABCD2, 88%; similarity with ALDP) codes for another peroxisomal transporter (ALDRP), that can be upregulated in rodent liver via PPARa nuclear receptor signalling. Our working hypothesis is that ALDRP could play a similar biochemical function, and could thus act as a modulator of X-ALD phenotype. Stable overexpression of the ALDRP by transgenesis in the ALD-deficient mouse background leads to full correction of VLCFAs in target organs and correlates with an improvement of the neurological phenotype in mice, a fact that turns ABCD2 into an interesting target for pharmacogenomics approaches to therapy. In parallel experiments, we have created an ALDR KO mouse and double ALD/ALDR KO. Initial results suggest an accelerated neurological phenotype in the double KO mice, but in absence of signs of cerebral demyelination. Thus, additional genetic or environmental factors may be required to initiate cerebral inflammatory demyelination in mice.

Program Nr: 189 from 2002 ASHG Annual Meeting

**Two mosaics for COL1A1 mutations causing Type III and IV Osteogenesis Imperfecta have a high percent of mutant bone cells: Implications for cell/gene therapy.** *W.A. Cabral, J.C. Marini.* SCTD, HDB/NICHD/NIH, Bethesda, MD.

Some parents of children with severe to lethal OI are germline and somatic mosaics for collagen mutations that cause severe disease in their children. The percent of mutant cells varies among different tissues of the same individual, making it impossible to extrapolate from leukocytes to skeletal tissues. The unaffected phenotype of mosaic parents is frequently cited as the naturally occurring model for the potential benefits of cell therapy, despite lack of information about the proportion of heterozygous cells present in mosaic bone. We examined skeletal cells from two asymptomatic mosaic carriers with COL1A1 mutations, who have a high proportion of heterozygosity in fibroblasts. One 160 cm tall woman is a mosaic carrier for type IV OI. Her DEXA and bone histology were normal. The causative IVS 19 $G^{+1}\rightarrow C$ collagen mutation is present in 80% of fibroblasts and 75% leukocytes. By both labelled PCR and single cell PCR the mutation was found in 75% of osteoblasts cultured from her left side and 50% of cells from her right side. In successive passages, mutant cells were a stable percent of fibroblasts but there is some selection against mutant osteoblasts. A second woman was a mosaic carrier for a mutation causing severe type III OI. Rib and calvarial bone histology were normal. Her genomic deletion causes loss of a1(I) aa 604-639. The mutation was present in 45% of fibroblasts and 60% of leukocytes. DNA from autopsy tissues was PCR-amplified and compared to a standard curve prepared from DNA of her fully heterozygous son. The proportion of heterozygous cells in tissue was 40% in calvarium, 60% in lung, 65% in tracheal rings and 70% in aorta. Our data provides the first demonstration that individuals mosaic for collagen mutations causing moderate to severe OI may have significant proportions of mutant cells. A 40-75% burden of mutant cells is compatible with normal bone growth, density and histology. This data is encouraging for both cell therapy and mutation suppression approaches, as it demonstrates that the presence of 25-60% normal osteocytes can result in incorporation of enough normal collagen to shift the structural balance of ECM in skeletal tissue.

Program Nr: 190 from 2002 ASHG Annual Meeting

**Clinical, Biochemical, Molecular and Therapeutic Findings in Alkaptonuria (AKU).** *C. Phornphutkul[1], W. Introne[1], M. Perry[2], I. Bernardini[1], P. Anderson[1], D. Fitzpatrick[1], M. D'Souza[1], M. Huizing[1], Y. Anikster[1], L. Gerber[2], W. Gahl[1].* 1) HDB/NICHD, NIH; 2) Rehabilitation Medicine, NIH, Bethesda, MD.

AKU, described by Garrod as due to autosomal recessive inheritance 100 year ago, results from defective tyrosine degradation. We describe the clinical spectrum of 58 patients(33 male, 25 female, age 4 to 80), along with new molecular findings and evidence that nitisonone, an herbicide which blocks enzyme 4-OH phenylpyruvate dioxygenase, can be useful for treatment. Result: In 21% of patients, the diagnosis was made before 1 yr of age. Later diagnoses followed presentations with ochronosis and joint pain. 94% of patients older than 40 had back pain before age 40. Spine flexion, assessed by the Schober score, decreased with age ($p<0.0001$). 50% of patients had at least one joint replaced by age 55. A general health survey (SF 36) revealed a correlation between physical combined score and age ($p<0.0001$). The ochronosis was variable. Soft tissue involvement presented in 57% of patients. 50% of patients had renal stones by age 64, and 50% had cardiac involvement by age 54. ESR and urinary NTX telopeptides were elevated. Urine HGA levels were ~300 fold normal (0.4-12.4 g/24h). At least one mutation in the AKU gene was found in 85% of patients. 82 % of mutations were compound heterozygous; M368V was the most common. Twenty-two mutations were not previously reported. There was no mutation hotspot, and no genotype/phenotype correlation. One patient was treated with very low dose (0.01-0.04 mg/kg/d) nitisinone. Urinary HGA declined from ~2.9 gm/d to 0.13 gm/d (>95% reduction). Plasma tyrosine levels rose from 60 mM to 719 mM on day 10, when the medication was discontinued. The patient remained asymptomatic. Conclusion: AKU should be considered a systemic disease that involves destruction of various tissues and significant morbidity. Most patients are compound heterozygous for AKU mutations. This may explain the clinical variability and the lack of obvious genotype/phenotype correlation. Low dose nitisinone lowers HGA production in human AKU patients and has the potential to cure this disorder. Further investigations are needed to assess the risks of hypertyrosinemia.

Program Nr: 191 from 2002 ASHG Annual Meeting

**Recombinant AAV-Mediated Gene Therapy for Phenylketonuria.** *C.E. Charron[1], L. Reyes[2], J.E. Embury[1,2], H.A. Steele[1], K. Ross[1], A.S. Lewin[3], T.R. Flotte[3,4], P.J. Laipis[1].* 1) Biochemistry and Molecular Biology, College of Medicine; 2) Department of Pathobiology, College of Veterinary Medicine; 3) Department of Molecular Genetics and Microbiology, College of Medicine; 4) Department of Pediatrics, College of Medicine; University of Florida, Gainesville FL 32610.

The only current treatment for Phenylketonuria (PKU) is dietary restriction of phenylalanine (Phe). While the characteristic mental retardation can be prevented by strict adherence to the medical diet, poor patient compliance is common, especially in adults. Gene therapy to deliver a functioning phenylalanine hydroxylase gene (PAH) to the liver would allow affected individuals to eat a normal diet. We have used a recombinant Adeno Associated Virus (rAAV) vector carrying the PAH gene to treat male *Pah*$^{enu2}$ mice, a missense mutation model for human PKU, via portal vein injection. The expressed PAH successfully lowered Phe serum levels for up to 36 weeks, the duration of the experiment. However a very high vector dose (0.7 to 1.5 x $10^{11}$ IU) was needed to observe a therapeutic effect, suggesting the possibility of dominant-negative interference by the endogenous missense PAH monomers. Evaluation of liver mRNA levels by Northern blotting showed abundant endogenous PAH in both sexes and all three genotypes. We are examining two alternate approaches to rAAV-based gene therapy in this animal model. First, a hammerhead ribozyme has been designed that targets the endogenous PAH gene to decrease the dominant-negative interference. This ribozyme has been tested *in vitro* on a 13-nucleotide RNA target; it cleaved 50 percent of the available target in 2 minutes at 5mM $MgCl_2$. The ribozyme sequence will be cloned into an rAAV vector and followed by an internal cleaving hairpin ribozyme to provide uniform 3’ ends on the mature ribozymes. Second, rAAV vectors either containing a Woodchuck Hepatitis Virus post-transcriptional regulatory element (WPRE) or rAAV vectors packaged with an alternate serotype of AAV coat protein with potentially higher liver targeting are being evaluated. The WPRE sequence has been shown *in vitro* to increase PAH activity by two-fold as compared to our original construct.

Program Nr: 192 from 2002 ASHG Annual Meeting

**Acute toxicity due to adenovirus versus cell-mediated immune response to the transgene in human argininosuccinate synthetase gene transfer.** *B. Lee[1], M. Guenther[2], J. Rodgers[2], V. Mane[1], W. O'Brien[1], A. Mian[1]*. 1) Molecular & Human Genetics; 2) Immunology. Baylor College of Medicine, Houston, TX.

Correction of urea cycle disorders requires high level hepatocyte transduction and intracellular transgene expression, making adenovirus a good vector for gene delivery. Systemic injection of a first generation adenoviral vector (FGV:CAG-hASS) expressing human argininosuccinate synthetase (hASS) in ASS-deficient calves was only transiently therapeutic (Lee *et al*. PNAS. 1999). We hypothesized that the short-term correction may have resulted from toxicity due to vector entry and viral gene expression, and/or from a cell-mediated immune response to viral antigens and perhaps the hASS protein, a potential neoantigen. In B6 mice injected with a high dose of FGV:CAG-hASS ($5x10^{12}$vp/kg), there was significant loss of hASS by week 4 post-injection, concomitant with liver enzyme elevation and liver architecture disruption. Rag2-/- immunodeficient mice treated with the same dose of FGV exhibited a decline in hASS expression and liver enzyme elevation comparable to that in B6 mice. Cytotoxic T-lymphocyte assays using a target cell line stably expressing hASS showed it to be poorly immunogenic in B6 mice. Hence, the Rag mice and CTL assay data taken together supported the interpretation that acute toxicity associated with vector entry and viral gene expression was probably the major causative factor for the decline in hASS expression. In contrast, mice injected with a helper-dependent adenoviral vector (HDV) expressing hASS (HDV:BOS-hASS) showed prolonged hASS expression. Furthermore, histological analyses showed absence of hepatic inflammation. In the absence of viral transcripts the HDV diminishes acute toxicity, further supporting our earlier interpretation. In conclusion, our data suggest toxicity associated with the FGV, rather than neoantigencity of the intracellular transgene, as an important determinant for gene transfer in urea cycle disorders in our animal models. Further development of HDVs for *in vivo* gene replacement for diseases such as urea cycle disorders is warranted due to a better toxicity profile and prolonged transgene expression.

Program Nr: 193 from 2002 ASHG Annual Meeting

**The iBAC: an efficient high capacity genomic DNA expression system.** *R. Wade-Martins, R. Innoue, K. Asadi-Moghaddam, M. Ranasinghe, E.A. Chiocca, Y. Saeki.* Neurosurgery Service, Massachusetts General Hospital, Charlestown, MA.

Gene transfer studies utilizing genomic DNA instead of complementary DNA deliver a transgene including the native promoter, the exons, introns and flanking regulatory regions. We have developed a genomic DNA expression system based on amplicon vectors carrying the $ori_S$ replication origin and the packaging/cleavage signal (*pac*) from Herpes simplex virus type 1 (HSV-1). These are excellent for genomic DNA delivery because they can carry a ~150 kb insert, are packaged by a helper virus-free system, and infect a wide range of cell types. We can deliver bacterial artificial chromosome (BAC) inserts >100 kb using our amplicon technology, termed the infectious BAC, or iBAC, vector. Previously, we demonstrated the efficient infectious delivery and expression of the 115 kb genomic DNA locus of the human *HPRT* gene (*Nature Biotechnology* 19:1067-1070). We now report the latest developments of our system. First, we are examining the ability of a genomic locus delivered by an iBAC to be correctly regulated by the same intracellular physiological signals as an endogenous chromosomal locus. We have efficiently expressed the human low density lipoprotein receptor (*LDLR*) locus by infectious delivery of the 130 kb locus in primary human fibroblasts from two patients with Familial Hypercholesterolaemia lacking functional LDLR. We are now quantifying sterol regulation of iBAC-*LDLR* expression. Second, we are using iBACs to express genes which possess complex splicing regulation. The *CDKN2A/CDKN2B* genomic region spans ~45 kb of DNA, contains six exons and encodes five genes from alternatively spliced, overlapping reading frames. Three genes from this region (*p15*, $p16^{Ink4a}$ and $p14^{ARF}$) are known negative regulators of cell division. We have shown expression of $p16^{Ink4a}$ and $p14^{ARF}$ from a BAC insert and hypothesize that delivery of a 132 kb insert of genomic DNA from this region using the iBAC system will result in correctly-spliced gene expression and restoration of cell cycle control in tumour cells. In summary, we believe the iBAC system represents a powerful approach for gene expression, suitable for linking genomic DNA sequence variation to gene expression and cell phenotype.

Program Nr: 194 from 2002 ASHG Annual Meeting

**Inherited gene correction of human mutations in purified CD34$^+$ cells directed by synthetic oligonucleotides.** *E.B. Kmiec, H. Parekh-Olmedo, L. Liu, M. Rice, E.E. Brachman.* Department of Biological Sciences, University of Delaware, Delaware Biotechnology Institute, Newark, DE.

The permanent correction of genetic mutations is the ultimate goal of many gene therapy approaches. Recently, [Yoon et al., PNAS, 93(5): 2071-2076] we introduced the concept of targeted gene repair, a process in which a synthetic oligonucleotide directs the repair of a chromosomal mutation. Since then, over 30 reports have confirmed and extended the technique, many of them in animal models. Correction of the mutation is directed by the oligonucleotide vector, which not only acts as a signal for the repair machinery, but also serves as a template for the nucleotide exchange. We have been elucidating the mechanism of targeted gene repair and have defined many of the enzymes and pathways that regulate the process (see Parekh-Olmedo and Kmiec 2002 and Brachman and Kmiec 2002 for recent reviews). By using *Saccharomyces* as a genetically tractable model system, many facets of the reaction, including strand bias, chromatin structure, and the identification of suppressor elements, have been revealed. Much of this information has been translatable into human cells, where the objective is to reverse a genetic mutation. We have altered the human globin gene at the specific nucleotide responsible for sickle cell anemia in human CD34$^+$ and Lin$^-$CD38$^-$ cells. The oligonucleotide vector was delivered either by microinjection or by electroporation, and gene repair determined by DNA sequencing at the genomic level and by mRNA production at the phenotypic level. These results establish, for the first time, directed gene repair of a human mutation in the globin gene in CD34$^+$ cells, and provide the template for the ultimate form of *ex vivo* human gene therapy.

Program Nr: 195 from 2002 ASHG Annual Meeting

**Cloning and engineering of entire mitochondrial genomes in *Escherichia coli* and transfer into isolated mitochondria.** *Y.G. Yoon, M.D. Koob.* Inst Human Genetics, Univ Minnesota, Minneapolis, MN.

The mitochondrial genome is essential for normal cellular energy metabolism, and mutations in this DNA molecule are known to cause a wide range of human diseases. Although the level of interest in these genomes is understandably high, no practical means have yet been found to directly modify mammalian mitochondrial sequences for either basic or applied research purposes. As a step towards overcoming this limitation, we have devised an efficient method for replicating and stably maintaining entire mitochondrial genomes in *E. coli* and have shown that we can then engineer these mtDNA genome clones using standard molecular biological techniques. In general, we accomplish this by inserting an *E. coli* replication origin and selectable marker into isolated, circular mtDNA at random locations using an *in vitro* transposition reaction and then transforming the modified genomes into *E. coli*. We tested this approach by cloning the 16 kb mouse mitochondrial genome, and found that the resulting clones could be engineered and faithfully maintained when we used *E. coli* hosts that replicated them at fairly low copy numbers. When these recombinant mtDNAs were replicated at high copy numbers, however, mtDNA sequences were partially or fully deleted from the original clones. We successfully electroporated recombinant mouse mtDNA into isolated mouse mitochondria devoid of their own DNA and detected *in organello* RNA synthesis by RT-PCR. This approach for modifying mtDNA and subsequent *in organello* analysis of the recombinant genomes should be applicable to a wide array of research studies and is a first step towards true in vivo engineering of mammalian mitochondrial genomes.

Program Nr: 196 from 2002 ASHG Annual Meeting

**Comparative Genomics of Human Chromosome 21: The Majority of Evolutionary Conserved Regions Are Not Genes.** *E.T. Dermitzakis[1], A. Reymond[1], R. Lyle[1], N. Scamuffa[1], C. Ucla[1], S. Deutsch[1], B.J. Stevenson[2], V. Flegel[2], C.V. Jongeneel[2], S.E. Antonarakis[1]*. 1) Division of Medical Genetics, University of Geneva, Geneva, Switzerland; 2) Swiss Institute of Bioinformatics, and Ludwig Institute for Cancer Research, Lausanne, Switzerland.

Chromosome 21 (HC21) is the smallest human chromosome. Several diseases are associated with genes or genomic regions of HC21 including Down syndrome, the most common cause of mental retardation. Comparative sequence analysis between species gives the opportunity to identify functional conserved genomic features. We aligned the entire HC21q sequence with the homologous sequences from mouse chromosomes 16, 17 and 10 from the Celera mouse assembly with PipMaker. 3508 blocks of ³100bps in length and ³70% sequence identity were identified. 1246 blocks corresponded to exonic regions (known) and the remaining 2262 have unknown function. We used 4 methods to obtain homology-based gene predictions and we tested 123 of these predictions with RT-PCR in 22 cDNA pools from human tissues. We have evidence for only 2 transcripts, which suggests that most of the conserved blocks do not correspond to expressed sequences. Computational analysis in known and unknown conserved blocks reveals patterns of nucleotide changes that strongly suggest that the majority of the unknown conserved blocks are non-coding. We further investigated the pattern of nucleotide change in 220 of these conserved blocks by sequencing them in 10 additional species representing different mammalian groups. We find that a large fraction of these regions (>50%) are highly conserved in multiple mammals further supporting functional conservation. Using multiple species analysis we determined the direction of the substitutions, and we find a highly significant heterogeneous pattern of species-specific substitutions that cannot be explained by differential mutational pressure, which suggests species-specific selective constraints. We argue that the majority of unknown conserved sequences identified on HC21 are not expressed and may represent regulatory regions or other structural features of the genome that contribute to common or unique species characteristics.

Program Nr: 197 from 2002 ASHG Annual Meeting

**Recent and divergent evolutionary origin of the segmental duplications flanking the 7q11.23 Williams-Beuren syndrome deleted region in hominoids.** *A. Antonell[1], O. de Luis[1], X. Domingo-Roura[2], L.A. Pérez Jurado[1]*. 1) Unitat de Genètica i; 2) Unitat de Biologia Evolutiva, Universitat Pompeu Fabra, Barcelona, Spain.

Williams-Beuren syndrome is a segmental aneusomy syndrome that results from a heterozygous deletion of a 1.5 Mb region at 7q11.23. Three large (~400 kb) region-specific segmental duplications or low-copy repeat elements (LCRs) composed of different blocks (called A, B and C) flank the deletion interval and predispose to misalignment and unequal crossing-over causing the deletions. Additional related segmental duplications exist in 7q11.23 and other chromosome 7 locations. FISH and zooblotting studies have shown that these blocks are single copy in mouse and other mammals but may also constitute LCR elements in other primates. In order to determine the evolutionary steps that led to this complex genomic structure we have studied the regional architecture in several primate species by sequence analysis, STR typing, and PCR of inter-block junction fragments. The orthologous region in macaque appears identical to that in mouse, with absence of local LCRs and presence of single copy sequences corresponding to the ancestral blocks B and C. In orangutan, a total of three copies of block C appear to exist, while blocks B and A are single copy. An additional block A but only two blocks C and the single ancestral block B are found in gorilla. Finally, block B sequences are still unique in chimpanzee, but this species has two blocks A, three blocks C and another segmental duplication not present in humans. Interestingly, Alu repeat elements were found at the junctions between LCR blocks. Our data indicate a very recent evolutionary origin of the 7q11.23 LCRs beginning just before the hominoid speciation dated 12 to 16 million years ago, with rapid but divergent proliferation thereafter in each species. The finding of Alu sequences at the inter-block junctions suggests that they might have facilitated the local rearrangements. The extraordinary rate of evolutionary turnover of the chromosome 7 segmental duplications results in important genomic variation among hominoid species which could be of functional relevance as well as predispose to disease.

Program Nr: 198 from 2002 ASHG Annual Meeting

**Computational Approaches for Performing Multi-Species Comparative Sequence Analysis: A Platform for Targeted Studies of Genome Architecture, Function, and Evolution.** *E.H. Margulies, NISC Comparative Sequencing Program, E.D. Green.* National Human Genome Research Institute, Bethesda, MD.

Deciphering the information coded within the human genome is a challenging task that will have tremendous benefits for all aspects of scientific research. Current efforts to annotate the human genome involve comparative analyses with a handful of other genome sequences (e.g., mouse, rat, and pufferfish). We are interested in developing more powerful approaches for comparative sequence analysis that utilize data generated from a larger number of species. As a complement to the various ongoing efforts to sequence entire vertebrate genomes, we now have in place a robust pipeline for mapping and sequencing the same targeted genomic region from multiple vertebrates in parallel, which to date includes chimpanzee, baboon, dog, cat, cow, pig, mouse, rat, chicken, two species of pufferfish, and zebrafish.

The data we are generating provides myriad opportunities for comparative sequence analyses. For example, sequences from each of the species are being examined for architectural features of interest (e.g., GC content, CpG islands, dinucleotide signatures), in many cases revealing compositional characteristics for each vertebrate. In addition, the general patterns of sequence conservation are being assessed, revealing important insights about the general extents of sequence conservation among different species and among different genomic regions. Finally, these data constitute a unique resource for delineating and measuring the complete spectrum of mutational events that shape the mammalian genome.

In summary, our approach for targeted sequencing in multiple species provides a spectacular opportunity for evolutionarily deep genome exploration. The resulting data is catalyzing the development of new computational tools and approaches for comparative sequence analysis, is providing intriguing new insights about genome function and evolution, and is yielding interesting information about the genomic regions being studied and the genes that reside therein.

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Program Nr: 199 from 2002 ASHG Annual Meeting

**ATLAS reveals extensive human genetic variability associated with L1 retrotransposons.** *R.M. Badge[1], R.S. Alisch[2], J.V. Moran[2].* 1) Genetics, University of Leicester, Leicester, United Kingdom; 2) Human Genetics, University of Michigan Medical School, MI.

ATLAS (amplification typing of L1 active subfamilies) is a PCR based genomic screening method that selectively amplifies the termini of young L1s and flanking genomic sequences. Use of primers directed to active L1 subfamily specific sequence variants ensures strong enrichment. High resolution gel electrophoresis enables the display of hundreds of amplicons that can be identified by PCR isolation and sequencing. Flanking sequences can then be used to locate the insertion in the human genome working draft (HGWD) sequence. 19 insertions identified by 3' terminus specific ATLAS all showed high identity to active (Ta) subfamily L1s. The flanking sequences of 8 L1s were located in the HGWD sequence, of which 3 were novel insertions. 5' terminus specific ATLAS was then used to isolate 48 amplicons from full length L1s. As expected, 25 correspond to either known (10) or novel (15) polymorphic insertions of the most active L1 subfamily, Ta1d. Ten of these loci were typed by genomic PCR in worldwide populations to confirm their insertional polymorphism. Seven insertions, all of which are absent from the HGWD sequence, are being tested in a cell culture retrotransposition assay. At least one of these elements is active, demonstrating the ability of ATLAS to discover novel elements that can jump in human cells. In addition, while it was suspected that the Ta1d specific variant utilised might occur in other L1s, 23 of these loci contain primate specific full length L1s, which are unlikely to show insertional polymorphism in humans. We present evidence that their polymorphic appearence is due to ATLAS' exquisite sensitivity to SNPs (at the L1 primer site, or the genomic restriction site). In conclusion ATLAS enables the rapid discovery of genome wide human genetic variation associated not only with young L1 subfamily insertional polymorphism, but also the abundant SNPs within old, fixed L1s. Full length L1 specific ATLAS also provides a means to screen human populations for L1 retrotransposons that are absent from the HGWD sequence and to characterise their retrotranspositional activity.

Program Nr: 200 from 2002 ASHG Annual Meeting

**Applied genomics: Exploring functional variation and gene expression.** *M.D. Adams[1], M.A. Cargill[1], E.G. Spier[2], F.M. De La Vega[2], S.J. Olson[2], T.J. White[3], J.J. Sninsky[3], D.A. Gilbert[2], M.W. Hunkapiller[2].* 1) Celera Genomics, Rockville, MD; 2) Applied Biosystems, Foster City, CA; 3) Celera Diagnostics, Alameda, CA.

The availability of the human genome sequence has presented researchers with tremendous opportunities to study the molecular basis for normal and pathogenic physiology with unprecedented completeness. Two common tools that have been used in these studies are genetic analysis identification of mutations or polymorphisms that affect gene function, and gene expression analysis of the set of genes active under a given set of cellular conditions. We have sought to improve both of these tools by 1) developing a very large set of validated single nucleotide polymorphism (SNP) assays, with an emphasis on gene-based SNPs, and 2) reagents for quantitative measurement of the expression analysis of each human gene. Both SNP and gene expression assay sets have been built using the TaqMan®system. To augment the set of potentially functional SNPs that are available for study, we are re-sequencing the exons, adjacent intron regions, and selected potential regulatory regions of all annotated human genes in 39 individuals from the Coriell human genetic cell repository as well as a chimpanzee. Prioritization of the potential regulatory regions will employ mouse-human synteny and motif scoring. Results to date indicate that about half of the SNPs that have been found had not been previously described. Most genes have at least one SNP predicted to cause a protein-coding change. Over 100,000 SNP-detection assays using endpoint TaqMan® allelic discrimination assays have been designed, synthesized, and validated in 90 DNA samples representing several human populations. These assays are designed to cover the genic-regions of the genome as well as to enable whole-genome scans and linkage disequilibrium studies as well as to have high heterozygosity in at least one major population. To complement this set of SNP assays, we are developing validating TaqMan®reagents for quantitative measurement of gene expression for over 14,000 RefSeq genes. This set will ultimately encompass all annotated human genes.

Program Nr: 201 from 2002 ASHG Annual Meeting

**High-throughput screening of allele frequency differences between DNA pools of cases and controls using four primer extension genotyping technologies.** *K.L. Mohlke[1], K.R. Wiles[1], J. Wilkowski[2], A.U. Jackson[3], K. Silander[1], M.R. Erdos[1], P.E. Bennett-Baker[2], L.J. Scott[3], T.E. Fingerlin[3], J. Wasson[4], M.A. Permutt[4], D.T. Burke[2], M. Boehnke[3], F.S. Collins[1]*. 1) NHGRI, NIH, Bethesda, MD; 2) Department of Human Genetics and; 3) Department of Biostatistics, Univ Michigan, Ann Arbor, MI; 4) Washington Univ School of Medicine, St. Louis, MO.

To facilitate positional cloning of complex trait susceptibility loci, we are investigating methods of genotyping DNA pools to scan single nucleotide polymorphisms (SNPs) for association. We examined four primer extension methods for SNP frequency analysis, including analysis by mass spectrometry (MS), pyrosequencing (PY), and fluorescent primer electrophoresis, which we tested on gel (FP1) and capillary (FP2) sequencers. For all methods we evaluated the precision of 8-16 replicate genotypes per SNP per pool and the accuracy of predicting allele frequency differences between DNA pools, using high-throughput design, handling and scoring, as possible. We genotyped 16 SNPs, with minor allele frequencies from .026 to .462, in three DNA pools of 499, 228 and 182 controls or cases of type 2 diabetes. The expected allele frequency differences between pools based on individual genotypes ranged from .001 to .118. The average absolute error in predicting allele frequency differences between pools was similar for MS (.009±.008), FP1 (.009±.008), PY (.011±.013) and FP2 (.012±.010). The precision, as measured by the average standard deviation, was slightly better for FP1 (.009±.004) and FP2 (.011±.008) compared to PY (.015±.012) and MS (.021±.010). This improved precision of FP over MS enables us to perform half as many replicates with comparable power and false positive results, although currently MS offers the highest throughput assay design and scoring. Compared to experimental variability, these sample sizes had a 2 to 3-fold greater effect on variability of frequency difference estimates between cases and controls from a population. Based on these data, we conclude that MS, FP and PY and pool sizes of 500 individuals can be used reliably to detect allele frequency differences of .07 or greater, providing useful tools to scan SNPs for association.

Program Nr: 202 from 2002 ASHG Annual Meeting

**Large-scale genotyping of complex DNA.** *G. Kennedy, H. Matsuzaki, S. Dong, M. Cao, W. Chen, J. Zhang, W. Liu, S.P.A. Fodor, K.W. Jones.* Genotyping Research, Affymetrix, Santa Clara, CA.

There is great interest in conducting studies to elucidate the genetic basis for complex diseases. Such studies are estimated to require genotyping of 10,000-500,000 genome-wide single-nucleotide polymorphisms (SNPs) in hundreds, and possibly thousands, of individual DNA samples. Current genotyping assays require locus-specific oligonucleotides and are limited to scoring SNPs in a serial fashion. Here we describe a simple but powerful method, called whole-genome amplification (WGA) for highly parallel genotyping of complex DNA. WGA uses generic primers for amplifying representative subsets of the human genome. Human genomic DNA sequence containing >185,000 SNPs has been synthesized by photolithography on high-density DNA microarrays, and SNPs genotyped by hybridization to allele-specific probes. We studied six genomic subfractions using this generic biochemistry. The method amplifies highly reproducible fractions of the genome across multiple DNA samples, achieves >99% accuracy, and is therefore well-suited to large mapping studies. We used this approach to rapidly determine the allele frequencies of 10,601 SNPs in three major human populations and to study geographic structure.

Program Nr: 203 from 2002 ASHG Annual Meeting

**A SNP linkage panel genotyped at ~ 1,000-plex on randomly assembled arrays.** *M.S.T. Hansen, A. Oliphant, J.-B. Fan, R. Shen, L. Zhou, B. Kermani, S. Kruglyak, T. Dickinson, C. Zhao, S. Barnard, D. Che, K. Gunderson, D. Barker, M.S. Chee.* Illumina, Inc., San Diego, CA.

A single nucleotide polymorphism (SNP) linkage panel, currently comprising over 2,000 SNPs, has been developed for highly multiplexed genotyping on randomly assembled arrays. The BeadArrayTM genotyping system, capable of processing hundreds of samples per day, has been validated in a production environment. The system is based on miniaturized arrays of beads located on the ends of optical fiber bundles. The arrays are formatted into a matrix that matches a 96-well microtiter plate, allowing samples to be processed in parallel using conventional robotic automation. A single instrument can read many such matrices per day. The capacity of an individual array is such that every assay in the array is performed redundantly, which increases robustness and measurement precision.

The SNP assay is highly multiplexed. Currently, the SNP linkage panel is processed at ~1,000-plex from beginning to end. Genotyping calls are made automatically, and each genotyping call is assigned a quantitative score that is correlated with quality. With a small number of robots and thermal cyclers, and a team of 5 people, we have the capacity to perform over one million genotypes per day. The system has the capacity to complete large genotyping studies in a short amount of time and at low cost. We will present the physical and genetic maps for the linkage set, together with key genotyping performance characteristics, including accuracy. The set can be deployed quickly, is cost effective, and is readily expandable.

Program Nr: 204 from 2002 ASHG Annual Meeting

**Genotyping error detection through tightly linked markers.** *G. Zou, D. Pan, H. Zhao.* Department of EPH, Yale University, New Haven, CT.

The identification of genotyping errors is an important issue in the identification of complex disease genes. Because it is common practice now to genotype multiple tightly linked genetic markers in a candidate region in genetic studies, we discuss genotyping error detections for a set of tightly linked markers in nuclear families in this article. We make use of the fact that recombination is a very unlikely event among tightly linked markers. We first show that, with family trios, no extra information can be gained from multiple tightly linked markers if there is no phase information available, and error detection rates are usually low if Mendelian consistency is used as the only standard for checking errors for family trios. However, for nuclear families with more than one child, error detection rates can be greatly increased with increase in the number of tightly linked markers. Error detection rates also increase with the number of children in each family. Because families displaying Mendelian consistency may still have genotyping errors, we calculate the probability that a family displaying Mendelian consistency has correct genotypes. These probabilities may potentially identify families that show Mendelian consistency but still have genotyping errors. In addition, we examine the benefit of complete linkage disequilibrium information in genotyping error detections. In one special case we considered, both error detection rates and the probability that an observed family displaying Mendelian consistency has correct genotypes are greatly increased in the presence of such additional information.

Program Nr: 205 from 2002 ASHG Annual Meeting

**Genotyping Error Introduces Bias to the Transmission Disequilibrium Test (TDT).** *A.A. Mitchell, D.J. Cutler, A. Chakravarti.* Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

The transmission disequilibrium test (TDT), a family-based test for linkage and association, is a popular tool in studies of complex inheritance, as it is simple to understand, simple to apply and robust to population stratification. We have determined that undetected genotyping errors can inflate the type I error rate of the TDT. This inflated error rate is due to a systematic bias in the TDT statistic in the presence of undetected genotyping error. We present a mathematical model which allows quantification of the size of this effect. Exploration of the model reveals several significant patterns in the magnitude and direction of the bias: 1.) Alleles of unequal frequency appear to be transmitted in a biassed manner. 2.) The apparent bias is non-random: a common allele appears to be overtransmitted to affected children, seeming to increase susceptibility. 3.) Increasing the number of trios linearly increases the bias magnitude. 4.) The fraction of genotyping errors detectable by Mendelian inheritance checking in trios is approximately 30%, but can be higher or lower, depending on the number and frequency of the alleles at the marker locus. These trends, predicted by our model, are apparent in the literature, indicating that many reported TDT-derived associations between diseases and marker alleles are likely to be false positives. Among studies that have used the TDT to demonstrate a significant association between a microsatellite allele and a disease, allele frequency is a significant predictor of whether the allele will appear over transmitted (OR = 26.9, p = 0.02). The same trend is true for SNPs, but the association is not significant (OR = 5.8, p = 0.44) perhaps due to lack of power.

Program Nr: 206 from 2002 ASHG Annual Meeting

**An Extended Analysis of Haplotype Structure in the Human Genome.** *S.B. Gabriel[1], S.F. Schaffner[1], D.E. Reich[1], P. Sabeti[1], M. Freedman[1], M.J. Daly[1], D. Altshuler[1,2].* 1) Center for Genome Research, Whitehead Institute, Cambridge, MA; 2) Departments of Genetics and Medicine, Harvard Medical School; Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

To systematically characterize human haplotype structure we have studied in detail 54 regions distributed across human chromosomes, genotyping a high density of markers in a large and diverse sample: 400 chromosomes drawn from European, Asian, African and African-American samples. An initial analysis of this data was published earlier this year (Gabriel et al. 2002, Science), describing four features of human haplotype structure: (a) the human genome can be objectively parsed into haplotype blocks: regions over which low rates of historical recombination are observed; (b) haplotype blocks are regions of low haplotype diversity, with 3-5 common haplotypes representing 90% of all chromosomes ; (c) haplotypes and block boundaries are highly conserved across populations; and (d) within blocks, the vast majority of markers show strong correlation ($r^2 > 0.5$) to the haplotype framework. In the present abstract we present an extended analysis of the the data, focused on two issues. First, we present a comparative analysis of the X chromosome and the autosomes, finding that population structure and linkage disequilibrium are dramatically different on the X chromosome as compared to the autosomes. These results have implications for the interpretation of previous surveys that (for technical ease of haplotype determination) used the X chromosome as a model for studying human diversity. Second, we present an analysis of haplotype sharing as a function of allele frequency, demonstrating a greater extent of linkage disequilibrium around alleles of lower frequency (<5%). These extended haplotypes will provide additional power for the study of rarer alleles, and suggest that the human haplotype map will be of broad utility under a range of assumptions about the allele frequencies of causal SNPs.

Program Nr: 207 from 2002 ASHG Annual Meeting

**Haplotype structure and linkage disequilibrium in 26 worldwide populations.** *D.E. Reich[1], S.B. Gabriel[1], D.B. Goldstein[2], G.M. McDonald[1], J.F. Wilson[2], P.C. Sabeti[1], M.G. Thomas[2], N. Bradman[2], R. Ward[3], R. Ferrell[4], A.-R. Linares[2], L. Pearce[5], B. Henderson[5], L.B. Jorde[6], D. Altshuler[1,7], E.S. Lander[1,8].* 1) Whitehead Institute, Cambridge, MA; 2) Univ. College, London, UK; 3) Univ. of Oxford, UK; 4) Univ. of Pittsburgh, PA; 5) USC, Los Angeles, CA; 6) Univ. of Utah, Salt Lake City; 7) Mass. General Hospital, Boston; 8) MIT, Cambridge, MA.

Linkage disequilibrium (LD) refers to correlations among neighoring alleles, reflecting 'haplotypes' descended from single, ancestral chromosomes. It is a major tool for gene mapping, and the extent of LD determines what density of markers will be necessary to carry out screens for alleles contributing to common disease. In two recent studies, we showed:

(1) LD around common alleles in genes extends long distances (tens of kilobases). This is almost an order of magnitude further than would be predicted under a model of history in which the human population expanded dramatically ~100,000 years ago. The long-range LD is due at least in part to a population bottleneck that occurred after the separation of European from African populations (Reich et al. 2001, Nature 411:199-204).

(2) The LD patterns in the human genome can be explained objectively in terms of haplotype blocks: sizeable regions over which there is little evidence for historical recombination, and within which only a few common haplotypes are observed. In north Europeans and east Asians, the typical block size is 22 kb, while it is 11kb in west African populations, underlying African populations' shorter extent of LD (Gabriel et al. 2002, Science, May 23).

To generalize these results to the range of human diversity, we genotyped 26 worldwide populations (more than 1,750 DNA samples) at 633 of the SNPs from the regions in study #1. We answer the question of whether a proposed haplotype map built in just three populations--a single west African, east Asian, and European one--would be equally useful in all groups, not just those in which the map was built.

Program Nr: 208 from 2002 ASHG Annual Meeting

**Haplotype structure in the mouse.** *M. Daly[1], C. Wade[1,2], A. Kirby[1], E.J. Kulbokas[1], J. Mulliken[3], E. Lander[1], K. Lindblad-Toh[1]*. 1) Whitehead Institute, Cambridge, MA; 2) University of Queensland, Brisbane, QLD, Australia; 3) Sanger Institute, Cambridge, UK.

Variable sites (SNPs) have previously been shown to be distributed non-randomly (Lindblad-Toh, 2001) among the commonly used lab strains of mice, but the structure of variation underlying this non-randomness has not been explored. We present an analysis of the fine structure of variation using data from both long contiguous published sequence and genome-wide shotgun SNP discovery. When the recently sequenced B6 genome is compared with data from other strains, we find long (multi-megabase) regions of either extremely high SNP rate (on average, 1 SNP per 200-250 bp) or extremely low SNP rate (on average, 1 SNP per >20,000 bp). In a comparison between 129 and B6, roughly one third of the genome falls into long high SNP rate region and this one third contains about 95% of the differences between the two strains. This higher polymorphism rate is consistent with estimated divergence rates between domesticus and musculus or castaneus. The regions of low and high SNP rate are shown to be consistent with long stretches of similarity or discordance of SSLP alleles from existing genetic map data. Furthermore, when many lab strains are compared locally, nearly every strain contains one of only two distinct haplotypes which (as expected from the individual strain to strain results) persist over long distances. Thus each commonly used lab strain has the appearance of being a different RI-like mosaic of an extremely limited founder stock. These observations have a dramatic impact on how these strains can be used in positional cloning experiments.

Program Nr: 209 from 2002 ASHG Annual Meeting

**The architecture of linkage disequilibrium blocks across Human chromosomes 6, 21, and 22.** *F. De La Vega, X. Su, H. Avi-Itzhak, C. Scafe, E. Spier.* Applied Biosystems, Foster City, CA.

With the aim of developing a linkage disequilibrium (LD) SNP map to serve as a resource for candidate-gene, candidate-region, and whole-genome association studies, we selected >230,000 SNPs to genotype on 90 DNA samples (45 African-American, 45 Caucasian, unrelated) selected from the Coriell Human variation collection. Our goal was to define a set of about 150,000 validated 5' nuclease assays distributed across all the genes in the genome for SNPs of high heterozygosity in at least one population. Our SNP selection for the set resembles a gene-focused picket fence with an average density of one SNP per 10 kb of gene length. The individual genotypes thus generated have enabled us to identify blocks of LD and to survey haplotype diversity across all gene regions of the Human genome for these populations. Here we present a comparative study of the architecture of the LD blocks across three entire Human autosomes: Chromosomes 6, 21, and 22. We selected for the study a total of 9,487 SNPs overlapping 2,266 predicted gene regions, with a minor allele frequency greater than 10% in either population, and that were in Hardy-Weinberg equilibrium ($p>0.01$). LD blocks were defined by requiring that all pairwise D' values of consecutive markers were >0.9 with statistical significance $p<0.001$ by the Fishers exact test. If these conditions were not met, this was interpreted as evidence of historical recombination. We identified a total of 651 LD blocks with an average length of 21.7 kb in the African-American population, whereas 1089 LD blocks averaging in length 31.9 kb were found in the Caucasian population. Blocks were 61% larger on average in chromosome 6 as compared with 21 and 22, the largest blocks being of 474 kb in length. Haplotypes were computationally inferred for the markers within each block by a variant of the EM algorithm, finding limited haplotype diversity (mean=4.2 per block). The distribution of block size and haplotype diversity across the chromosome axis was not random. We analyze the relationship of these metrics with other chromosomal features, including base composition, recombination rate, abundance of repetitive elements, and gene density.

Program Nr: 210 from 2002 ASHG Annual Meeting

**A First Generation Haplotype Map of Chromosome 19.** *M.S. Phillips[1], R. Sachidanandam[2], M.A. Donaldson[1], G.R. Abecasis[3], R. Lawrence[4], J.F. Studebaker[1], W.M. Ankener[1], F.-S. Kuo[1], S.V. Alfisi[1], G.A. Gelfand[1], M.T. Boyce-Jacino[1], L.R. Cardon[4].* 1) Orchid BioSciences Inc, Princeton, NJ; 2) Cold Spring Harbor Laboratories, NY; 3) University of Michigan, Ann Arbor, MI; 4) Oxford University, Oxford, UK.

Identifying regions of chromosome conservation in the human genome is essential for detecting and characterizing genetic predictors of common multifactorial disease and for the advancement of personalized diagnostic, prognostic and therapeutic medicines. Accordingly, a large-scale international initiative to construct haplotype maps of the human genome has been launched as the natural follow-on to the Human Genome Sequence Project. The project aims to distinguish between chromosomal regions that are ancestrally conserved amongst individuals and those which have been disrupted due to likely forces of recombination, mutation and selection.

To date, first-generation haplotype maps have been constructed for chromosomes 21 and 22, both of which were built on finished DNA sequence. Despite substantial design differences in these studies: involving wide-scale characterization of LD in many Caucasian samples (chr22) vs fine-scale haplotype definition in few diverse ethnicities (chr21), the results suggest that some surprisingly large chromosome regions have been ancestrally conserved, while others seem prone to a high rate of rearrangement.

Here we construct a haplotype map of chromosome 19 using 3783 robust SNP markers, which we have analyzed on CEPH caucasian family samples. These markers comprise a ~14 kb (median) map of the chromosome. We further genotyped a subset of the above markers on population samples (Caucasian, African American and Asian populations) that were used for SNP validation in the SNP Consortium allele frequency project in order to compare the Caucasian/CEPH patterns of LD with those of diverse ancestry. Initial haplotypic analysis of the chromosome is revealing a complex pattern of LD regions that are conserved across populations.

Program Nr: 211 from 2002 ASHG Annual Meeting

**A haplotype map of Chromosome 19.** *M.T. Boyce-Jacino[1], R. Sachidanandam[2], M.A. Donaldson[1], G.R. Abecasis[3], R. Lawrence[4], J.F. Studebaker[1], W.M. Ankener[1], F.S. Kuo[1], S.V. Alfisi[1], C.A. Gelfand[1], M. Phillips[1], L.R. Cardon[4].* 1) Orchid Biocomputer, Inc, Princeton, NJ; 2) Cold Spring Harbor Laboratories, NY; 3) University of Michigan, Ann Arbor, MI; 4) Oxford University, Oxford, UK.

Identifying regions of chromosome conservation in the human genome is essential for detecting and characterizing genetic predictors of common multifactorial disease and for the advancement of personalized diagnostic, prognostic and therapeutic medicines. Accordingly, a large-scale international initiative to construct haplotype maps of the human genome has been launched as the natural follow-on to the Human Genome Sequence Project. The project aims to distinguish between chromosomal regions that are ancestrally conserved amongst individuals and those which have been disrupted due to likely forces of recombination, mutation and selection. To date, first-generation haplotype maps have been constructed for chromosomes 21 and 22, both of which were built on finished DNA sequence. Despite substantial design differences in these studies: involving wide-scale characterization of LD in many Caucasian samples (chr22) vs fine-scale haplotype definition in few diverse ethnicities (chr21), the results suggest that some surprisingly large chromosome regions have been ancestrally conserved, while others seem prone to a high rate of rearrangement. Here we construct a haplotype map of chromosome 19 using 3783 robust SNP markers, which we have analyzed on CEPH caucasian family samples. These markers comprise a ~14 kb (median) map of the chromosome. We further genotyped a subset of the above markers on population samples (Caucasian, African American and Asian populations) that were used for SNP validation in the SNP Consortium allele frequency project in order to compare the Caucasian/CEPH patterns of LD with those of diverse ancestry. Initial haplotypic analysis of the chromosome is revealing a complex pattern of LD regions that are conserved across populations.

Program Nr: 212 from 2002 ASHG Annual Meeting

**Haplotype Map of Human Chromosomes 7 and X at 100 kb SNP Marker Density.** *P. Taillon-Miller[1], N.L. Saccone[2], S. Duan[1], E. Lovins[1], R. Donaldson[1], E. Vieux[1], J.P. Rice[2], P-Y. Kwok[3].* 1) Dermatology, Washington Univ Medical Sch, St Louis, MO; 2) Department of Psychiatry, Washington Univ Medical Sch, St Louis, MO; 3) Cardiovascular Research Institute and Department of Dermatology, University of California, San Francisco, San Francisco, CA.

There has been a great deal of interest lately in the proposal to develop a map of common haplotypes in the human genome. For several years now our group has been looking at linkage disequilibrium between common SNP markers. We have recently expanded this study to all of chromosomes X and 7. To accomplish this goal we have increased our genotyping capacity to >2.4 million genotypes a year using a 4 person team and plan to double this over the next several months using a 3 person team. For our project we used SNPs in which the minor allele frequencies are greater than 10 percent; in European, Asian, and African-American samples. We have utilized the >70,000 characterized SNPs from our group and others that are now publically available. We have designed PCR and SNP detection assays for all available SNPs so our current targeted SNP characterization efforts are based solely on SNPs in which assays have already been successfully designed. We are approaching 2000 SNPs that meet our criteria for both chromosome 7 and X with average marker density approximately 1/90 kb. To date we have genotyped close to half of the available markers for each chromosome. Our most recent analysis of 526 SNP markers from the X chromosome has revealed numerous new linkage disequilibrium blocks with reduced haplotype representation. More than 10 sequencing contiqs in this analysis had SNP marker density better than 1 SNP every 40 kb. Analysis of the chromosome 7 data is underway. The future goals will be to increase the density of the SNP markers. These data constitute a major step forward in the construction of whole chromosome haplotype maps.

Program Nr: 213 from 2002 ASHG Annual Meeting

**Variation across the X chromosome.** *D.J. Mathews, D.J. Cutler, M.E. Zwick, A. Chakravarti.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD.

SNPs are not uniformly distributed in the genome. To understand the patterns of variation and their implications for association studies, we present a study of ~50 kb of unique sequence from each of 8 ~100 kb X-linked regions encompassing the genes: Glycine Receptor Alpha-Subunit 2 (GLRA2), Monoamine Oxidase A (MAOA), K Channel, member 1 (KCND1), Alpha thalassemia/MR Sx (ATRX), Alpha-Galactosidase A (AGA), Transient Receptor Potential Channel 5 (TRPC5), Bombesin-like Receptor (BRS3) and Methyl CpG Binding Protein 2 (MECP2). We sequenced 40 human males from the Polymorphism Discovery Resource using chip-based resequencing and identified 48-119 putative SNPs per locus, for a total of 705.

The 8 loci were chosen to represent a range of variables such as coding versus noncoding sequence, recombination rate and GC content, and are distributed across the entire length of the X chromosome. Nucleotide diversity, p, ranges from 1.54-5.48 x $10^{-4}$ per base pair. None of the 8 loci have a significant Tajima's D value ($p < 0.05$). The rate of recombination varies considerably, with the proportion of sites for which all 4 gametes are present ranging from 0.277 in ATRX to 0.604 in MAOA, which is one of a pair of genes resulting from an ancestral duplication. Also of note is an inverse relationship between the number of SNPs in a given 10 kb bin and the number of singletons in that bin.

Neighbor-Joining phylogenetic analysis of the 8 regions, both individually and *en masse*, show remarkably different levels and patterns of population structure. In addition, when one constructs trees using sliding overlapping windows of 20 kb across a locus, some loci show more structure within windows than across the locus, and notably, *changing* structure across these 'allele' trees. In contrast, other loci show quite consistent structure across the region. This pattern of change has important implications for association studies, which have historically used ethnically or racially identified samples and matched controls. These data suggest that allelic history is important to such studies in outbred populations.

Program Nr: 214 from 2002 ASHG Annual Meeting

**Density and distribution of single nucleotide polymorphisms (SNPs) across the human genome.** *Z. Zhao, D. Hewett-Emmett, E. Boerwinkle.* Human Genetics Center, Univ Texas Houston, Houston, TX.

Single nucleotide polymorphisms (SNPs) are valuable tools for localizing and identifying disease susceptibility genes, understanding the molecular mechanisms of mutation, and deducing the origins of modern human populations. Discovery of SNPs has been carried out via surveys at the genome-level and concerted efforts focusing on specific genomic regions. In this study, we further investigated the SNP density and distribution across the human genome and in different genic categories using two SNP databases: Celeras RefSNP (version 3.2), which includes SNPs from a variety of sources and is biased toward disease associated genes, and Celeras CgsSNP, which includes SNPs identified by comparing genomic sequences and represents an unbiased survey of SNPs across the genome. We analyzed 3,580,926 SNPs with associated genome annotation information from RefSNP and 2,438,592 SNPs from CgsSNP. Based on RefSNP, the average numbers of SNPs per 10 kb was 12.23, 11.50, and 13.78 in the human genome, in the intergenic regions, and in the genic regions, respectively. Based on CgsSNP, these numbers were 8.33, 8.44, and 8.09, respectively. In the genic regions, the SNP density in the intronic, exonic and adjoining untranslated regions was 13.53, 18.15, and 17.79 SNPs per 10 kb, respectively, based on RefSNP, and 8.21, 5.28, and 7.51 SNPs per 10 kb, respectively, based on CgsSNP. For both databases, the distribution of SNPs in 10-kb interval was nonrandom and significantly different with the Poisson distribution. For example, the 10-kb intervals observed with less than 25 SNPs accounted 90% and 97% for RefSNP and CgsSNP, respectively. The number of SNPs per chromosome was correlated with chromosome length, but the density of SNPs estimated by CgsSNP was not significantly correlated with the GC content of the chromosome. Based on CgsSNP, both the ratio of nonsense to missense mutation (0.027) and the ratio of missense to silent mutations (1.15) were less than half of that expected values (0.059 and 2.93, respectively) under the neutral mutation theory, reflecting a role for natural selection, especially purifying selection.

Program Nr: 215 from 2002 ASHG Annual Meeting

**A high-resolution human SNP linkage map.** *T.C. Matise[1], R. Sachidanandam[2], A. Clark[3], L. Kruglyak[4], E. Wijsman[5], B. Chui[6], P. Cohen[7], C. deToma[7], M. Ehm[8], S. Glanowski[3], C. He[1], J. Heil[3], I. McMullen[3], L. Stein[2], M. Wagner[8], J. Winick[6], E.S. Winn-Deen[3], H.M. Cann[7], E. Lai[8], H.L. Holden[9]*. 1) Rutgers U., Piscataway, NJ; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; 3) Celera Genomics, Rockville, MD; 4) Fred Hutchinson Cancer Research Center, Seattle, WA; 5) University of Washington, Seattle, WA; 6) Motorola Life Sciences, Tempe, AZ; 7) Foundation Jean Dausset - CEPH, Paris, France; 8) GlaxoSmithKline, Research Triangle Park, NC; 9) First Genetic Trust, Deefield, IL.

The use of single nucleotide polymorphisms (SNPs) as genetic markers can provide extremely efficient genotyping. Utilizing the SNP resources generated by The SNP Consortium, we have designed a genome-wide linkage marker set with higher resolution, lower genotyping cost, and higher throughput than currently available microsatellite marker sets. We have constructed a linkage map with 2772 SNPs distributed as both singletons and clusters (groups of 2-4 SNPs spanning < 100kb). The effective map resolution is 4 cM. Over 6,000 SNPs were initially genotyped in 90 individuals from the TSC diversity panel (30 Caucasians, 30 African Americans, 30 Asians) in order to assess genotyping success rates, assay quality, population specific allele frequencies, multi-SNP haplotype heterozygosities, and levels of linkage disequilibrium. A subset of 2772 maximally useful SNPs were chosen for development of the linkage map. The average minor allele frequency was 0.3 in each of the three populations. The average multi-SNP (cluster) haplotype heterozygosity across all populations was 71% (71% in Caucasians, 73% in African Americans, 69% in Asians). The average degree of linkage disequilibrium between all pairs of SNPs in each 3-SNP cluster was $r^2$=0.1. The selected SNPs were genotyped in 661 individuals from 48 CEPH reference pedigrees. Meiotic linkage maps were constructed de novo, independent of any other mapping or sequence position information. The SNP linkage maps show 93% concordance with the NCBI Build 29 genome assembly (range 62-100%). This linkage set has greater information content than the current Marshfield screening set. Data and results are available at http://snp.cshl.org.

Program Nr: 216 from 2002 ASHG Annual Meeting

**Detection and mapping of HCHOLA4, the fourth gene involved in familial hypercholesterolemia.** *L. Villéger[1], M. Abifadel[1, 2], D. Allard[1], J.P. Rabès[1, 3], J. Weissenbach[4], M. Varret[1], C. Junien[1, 3], C. Boileau[1, 3].* 1) Hosp Necker - Enfants Malades, INSERM U383, Paris, France; 2) Faculté de Pharmacie, Université Saint-Joseph, Beirut, Lebanon; 3) Service de Biochimie, CHU Ambroise Paré, Boulogne, France; 4) Centre National de Séquençage, Evry, France.

Autosomal Dominant Hypercholesterolemia (ADH) is one of the most common hereditary diseases, characterized by a selective increase of LDL particles, giving rise to premature mortality from cardiovascular complications. ADH results from molecular defects in the LDLR gene (Familial Hypercholesterolemia), the APOB gene (Familial ligand-Defective apolipoprotein B-100) and the HCHOLA3 gene (formerly FH3, 1p32-p34.1). We identified a large French ADH family (HC6) in which the involvement of these 3 genes was excluded suggesting the existence of a fourth locus (HCHOLA4). We undertook the identification of this new locus using linkage analysis in family HC6 by a candidate region approach. After the exclusion of 30 candidates, we undertook a whole genome approach with 232 new microsatellite markers. Linkage was obtained with a Lod score of 3.86 (q=0) and confirmed by a multipoint Lod score analysis. Suggestive linkage was also obtained for 6 other nonLDLR/nonAPOB/nonHCHOLA3 families comforting the localization of the HCHOLA4 locus in this genomic region. Finally, a critical region of 1.9 cM was defined which contains the HCHOLA4 gene. We constructed a 3.8 Mb physical map covering our genetic region and identified 79 genes. The coding and exon/intron boundaries of 47 functional and positional candidates have been sequenced in collaboration with the Centre National de Squenage. Finally, major rearrangements in 10 regional and functional candidates have been investigated by Southern Blot. We have identified 52 SNPs, but no disease-causing variations. This new locus confirms the high degree of genetic heterogeneity of autosomal dominant familial hypercholesterolemia and underscores the existence of unknown aspects of cholesterol homeostasis.

Program Nr: 217 from 2002 ASHG Annual Meeting

**Identification of an FCHL-Associated Gene on Chromosome 1q21-q23.** *P. Pajukanta[1], M.-R. Taskinen[2], A. Kuraishy[1], J. Schmidt[1], A.J. Lusis[1], L. Peltonen[1,3].* 1) Dept of Human Genetics, UCLA, Los Angeles, CA; 2) Dept of Medicine, University of Helsinki, Finland; 3) Dept of Molecular Medicine, National Public Health Institute, Finland.

To assess the unknown genetic background of coronary heart disease (CHD), we investigated the most common dyslipidemia predisposing to CHD, familial combined hyperlipidemia (FCHL), affecting 1-2% of the Western populations and 10-20% of families with premature CHD. FCHL is characterized by elevated levels of serum total cholesterol, triglycerides, or both. We previously identified a novel locus for FCHL on human chromosome 1q21-q23 in Finnish FCHL families. This finding has been replicated in FCHL families from other, more heterogeneous populations and importantly, the same markers have also been linked to type 2 diabetes mellitus, the phenotype clearly overlapping with FCHL. We used 61 extended Finnish FCHL families with 914 family members with defined quantitative phenotype information. Analyses of the peak linkage markers showed that in the families contributing to linkage, 86.4% of affected individuals shared a haplotype in a 3-4 Mb region, the shared haplotype not being identical in all families. We genotyped a denser marker set of 40 microsatellites and SNPs in this region and monitored for LD and association of small regional haplotypes using family-based association analyses and the gamete competition test. We identified initial evidence of association ($P<0.005$) with SNPs over a 230 kb region between the JAM1 and APOA2 genes on 1q21-q23. A total of 16 genes or predicted transcript are assigned to this region. We genotyped additional SNPs for these genes and found significant evidence for association ($P<0.00001$) by combining several SNPs within one of the transcripts using the gamete competition test. The results of the multipoint linkage disequilibrium analysis (HRRMULT) also supported this finding. Both exons and introns of the associated transcript are currently under sequencing in 61 FCHL probands to identify all possible variants. This strategy enables us to test the statistical and functional significance of all potential changes to finally identify the causative FCHL variant(s).

Program Nr: 218 from 2002 ASHG Annual Meeting

***KLOTHO* allele status as a potent and environmentally modified risk factor for atherosclerosis.** *D.E. Arking[1], D.M. Becker[2], L.R. Yanek[2], D. Judge[2], T.F. Moy[2], L.C. Becker[2], H.C. Dietz[1, 3]*. 1) Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD; 2) Johns Hopkins Medical Institutions, Baltimore, MD; 3) HHMI.

We previously identified an allele of *KLOTHO*, termed KL-VS, that is prevalent in the general population (q = 0.16) and in homozygosity is associated with reduced human longevity. This allele is characterized by two amino acid substitutions in complete linkage disequilibrium that influence klotho metabolism. Klotho-deficient mice display extensive and accelerated atherosclerosis on a normal diet, suggesting a potent genetic predisposition. To determine whether klotho influences atherosclerotic risk in humans, we employed a population-based association study in a cohort of apparently healthy individuals (n=497, age=467, male=50%, African American=16%, current smokers=31%) with siblings that have been diagnosed with early-onset (< 60 years) coronary artery disease. KL-VS genotype frequencies are significantly different between individuals with and without occult atherosclerosis (defined as abnormal stress/thallium test) ($P < 0.006$). Univariate regression analysis assuming an additive model and incorporating a Generalized Estimating Equations (GEE) adjustment for family clustering indicates a 1.90-fold (95% C.I. 1.21-2.98) increased risk of disease conferred by the KL-VS allele ($P < 0.005$). Addition of a KL-VS genotype term into a General Linear Model (GLM) incorporating known risk factors (age, sex, race, BMI, HTN, HDL, LDL, TG, diabetes, and current smoking) significantly improved the model, indicating that the KL-VS allele is a novel risk factor for occult atherosclerosis ($P < 0.019$). A significant KL-VS allele/current smoking status interaction was observed ($P < 0.031$), and incorporation of this interaction term significantly improved the GLM ($P < 0.006$), indicating that the KL-VS allele confers a 3.37-fold (95% C.I. 1.11-10.22) increased risk of occult atherosclerosis in smokers. These data demonstrate a role for *KLOTHO* in early-onset atherosclerosis and identify a strong gene/environment interaction that confers a modifiable risk for a common disease with complex inheritance.

Program Nr: 219 from 2002 ASHG Annual Meeting

**Localization by genome scan of two large QTLs influencing HDL levels in FCHL pedigrees with exploration of their relationship to triglyceride levels.** *F. Gagnon[1,2], G.P. Jarvik[2], A.G. Motulsky[2], S.S. Deeb[2], J.D. Brunzell[2], E.M. Wijsman[2]*. 1) Department of Epidemiology and Community Medicine, University of Ottawa, Ottawa, ON, Canada; 2) Department of Medicine, University of Washington, Seattle, WA, USA.

Familial combined hyperlipidemia (FCHL) is a common and complex disease that is characterized by several lipid-related abnormalities including elevated plasma levels of triglycerides (TG) as well as reduced high-density lipoprotein (HDL). FCHL demonstrates a complex pattern of inheritance and to date, no genetic defects of major effect have been identified. Physiological and statistical relationships between plasma TG and HDL levels are well established. We completed a genome scan for HDL levels in 4 large (N=255) FCHL families ascertained through hypertriglyceridemic probands. The genotyping was done by the NHLBI Mammalian Genotyping service using 387 anonymous markers. Using covariated-adjusted (age and sex) joint linkage and segregation analysis based on Bayesian Markov chain Monte Carlo methods, we localized 2 large quantitative trait loci (QTLs) with individual contributions of ~ 27% and 11% of total HDL variance, respectively. One of these QTLs is located at the APOC3 region on chromosome 11. Three smaller QTLs were also identified. Adding TG as a covariate decreased 4 out of the 5 linkage signals, including the signal at the APOC3 locus. Joint analysis based on 90 markers of the chromosomes with positive linkage signals further supports linkage of the two large QTLs identified in the initial analyses, with intensity ratios for linkage, calculated for 2 cM intervals, of ~ 21 for the signal at the APOC3 locus and 45 for the other locus. The intensity ratio is the posterior acceptance rate of QTL positions in an interval to the prior such rate. Analyses of LN TG trait using the same joint chromosome analysis also revealed TG QTLs with intensity ratios of ~ 70 and 19 but in different locations. Using covariate adjustment and simultaneous analyses of chromosomes will be useful in fine mapping of genes implicated in FCHL.

Program Nr: 220 from 2002 ASHG Annual Meeting

**Consistent and Significant Associations between Hypertension and renin-angiotensin system genes in two populations.** *X. Zhu[1], C. Chang[2], D. Yan[2], A. Weder[3], R. Cooper[1], A. Luke[1], D. Kan[1], A. Chakravarati[2].* 1) Preventive Med & Epidemiology, Loyola Univ Medical Ctr, Maywood, IL; 2) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Univ., Baltimore, MD; 3) Division of Hypertension, Univ. of Michigan School of Medicine, Ann Arbor, MI.

The genes of the renin-angiotensin system (RAS) have been subjected to the most intense molecular scrutiny of any regulatory pathway involved in cardiovascular disease (CVD), although their contribution to CVD is still unclear. In this study we sampled 604 individuals from 192 African-American families and 608 individuals from 153 European-American families. We genotyped 25 single nucleotide polymorphisms in four genes: 10 in angiotensin-converting enzyme(ACE), 5 in angiotensinogen, 5 in angiotensin type I receptor (AGRT1), and 5 in renin. Hypertension was defined as individuals treated with antihypertensive medication and either SBP>120 or DBP >70, or as individuals without antihypertensive medication but with both SBP > 140 and DBP >90. Family-based transmission/disequilibrium tests were performed with each SNP in blacks and whites separately, and combined. The results demonstrate that REN4059 and AGTR1-1062 are significantly associated with hypertension in Blacks ($P<0.0006$), as well as in the pooled data ($p=0.02$ for REN4059 and $<0.00001$ for AGTR1-1062). Using haplotypes we defined in a previous study (Zhu et al. 2002), we conducted haplotype TDT analysis. Significant evidence was further confirmed in the haplotypes consisting of Ren513-Ren4059, AGTR1-573-1062 and ACE2400-10979-14521-18912 in Blacks; similar but weaker evidence was found in whites. We next randomly sampled one sibling from each family and obtained 84 cases and 108 controls in blacks, and 41 cases and 113 controls in Whitse. Single SNP based case-control analysis as well as haplotype analysis again showed results similar to those above. Because of different samples used, Consistent results from both analyses strengthen the evidence for association between hypertension and variants in these genes. Our analysis also demonstrates that block-based haplotype analysis can be a powerful method to examine complex traits such as hypertension.

Program Nr: 221 from 2002 ASHG Annual Meeting

**A Cysteinyl leukotriene 2 ($CysLT_2$) receptor variant associated with atopy in the population of Tristan da Cunha.** *M.D. Thompson[1], K. Storm[6], H. Galczenski[2], K.A. Siminovitch[3], N. Zamel[5], A.S. Slutsky[5], J. Drazen[6], J.F. Evans[2], S.R. George[1,4,5], B.F. O'Dowd[1,4]*. 1) Dept. of Pharmacology, University of Toronto, Toronto, ON, Canada; 2) Dept. of Pharmacology, Merck Research Labs, West Point, PA; 3) Dept. of Medical Genetics and Microbiology, University of Toronto, Toronto, ON, Canada; 4) Centre for Addiction and Mental Health, Toronto, ON, Canada; 5) Dept. of Medicine, University of Toronto, Toronto, ON, Canada; 6) Harvard Medical School, Boston, MA.

The heterogeneous nature of asthmas etiology suggests that a study of the contribution of genetic variability in candidate loci to well-defined phenotypes, such as atopy, may be fruitful in identifying genetic risk factors. The cysteinyl leukotriene 2 ($CysLT_2$) receptor is a candidate that relates to atopy for the following reasons: 1) the receptor is found within a 1 MB region on chromosome 13q14 shown to be associated with atopy; 2) the receptors functional role in mediating leukotriene pathways is implicated in the etiology of atopy; and 3) the differential $CysLT_2$ receptor mRNA expression in eosinophils is implicated in atopy. In view of this, the contribution of variability in the Cys $LT_2$ receptor gene within the population of Tristan da Cunha, characterized by a founder effect and a 47% prevalence of atopy, was studied. In order to study this variability, we used single stranded conformational polymorphism analysis and dideoxy sequencing to identify the wild type and four variants of the Cys $LT_2$ receptor. One, the Met202Val variant, was activated with four fold less potency, compared to the wild type receptor, by leukotriene $D_4$ ($LTD_4$). The Met202Val mutation is located on the cytoplasmic side of the putative fifth transmembrane spanning domain of the $CysLT_2$ receptor, a position that may disrupt ligand binding and result in altered agonist potency. The novel Met202Val $CysLT_2$ receptor variant was found at significantly higher frequencies (P=0.003) among the atopics (21%) compared with non-atopics (7%). These findings reveal an association between atopy in the Tristan da Cunha population and a functional mutation of the atopy candidate gene encoding the $CysLT_2$.

Program Nr: 222 from 2002 ASHG Annual Meeting

**Gene-gene and gene-environment interactions in the IL-4/IL-13 pathway in the development of asthma.** *D.L. Newman[1], D. Nicolae[2], S. Colilla[1], R. Nicolae[1], T.D. Howard[3], K.C. Barnes[4], M.N. Blumenthal[5], T.H. Beaty[4], S.S. Rich[6], R.A. King[5], E.R. Bleecker[3], N.J. Cox[1], D.B. Mirel[7], C. Ober[1] and Collaborative Study on the Genetics of Asthma (CSGA).* 1) Dept of Human Genetics, Univ of Chicago, Chicago, IL; 2) Dept of Statistics, Univ of Chicago, Chicago, IL; 3) Center for Human Genomics, Wake Forest Univ, Winston-Salem, NC; 4) Dept of Epidemiology, Johns Hopkins Univ, Baltimore, MD; 5) Dept of Medicine, Univ of Minnesota, Minneapolis, MN; 6) Dept Public Health Sciences, Wake Forest Univ, Winston-Salem, NC; 7) Dept Human Genetics, Roche Molecular Systems, Alameda, CA.

Asthma is a complex disease that is influenced by both genetic and environmental factors. Genes in the IL-4/IL-13 pathway have been extensively studied as candidates for asthma susceptibility. Previously we reported an interaction between exposure to cigarette smoke in infancy and evidence for linkage to the region on chromosome 5q31 that encodes IL-4 and IL-13 in 144 Caucasian families from the CSGA (Colilla et al., AJHG S69:213, 2001). Both of these cytokines share a common receptor alpha-chain, encoded by the IL4RA gene on 16p12. Here we investigate the relationship between cigarette smoke exposure in infancy and 11 polymorphisms in IL4, IL13 and IL4RA in 233 Caucasian trios and 298 Caucasian non-asthmatic controls ascertained by these same four CSGA centers. Probands were stratified by exposure (169 unexposed, 64 exposed) and the data were analyzed using both TDT (trios) and case-control approaches. There was significant nonrandom transmission of IL13 haplotypes to asthmatic children in the unexposed group ($p=0.016$), and the IL13 gln110 allele was modestly increased in this group compared with controls ($p=0.05$). No association was present in the exposed group. Furthermore, using a 2-locus TDT, we identified a significant interaction between the IL13 arg110gln and IL4RA polymorphisms ($p=0.001$), suggesting an interaction between this Th2 cytokine and its receptor. Overall these data highlight the importance of considering both gene-gene and gene-environment interactions in studies of complex diseases, and further implicate these genes in the pathobiology of asthma.

Program Nr: 223 from 2002 ASHG Annual Meeting

**Cohort effects and an age-adjusted genome screen for maximum number of drinks in the COGA dataset.** *N.L. Saccone[1], N. Rochberg[1], A. Goate[1], H. Edenberg[2], T. Foroud[2], T.-K. Li[2], T. Reich[1], H. Begleiter[3], J.P. Rice[1]*. 1) Washington University, St. Louis, MO; 2) Indiana University, Indianapolis, IN; 3) SUNY Health Science Center at Brooklyn, Brooklyn, NY.

Epidemiological studies have shown that rates of alcohol dependence in younger individuals exceed lifetime disease risks in older individuals, even though younger individuals are not yet through the period of risk. Analysis of the COGA (Collaborative Study on the Genetics of Alcoholism) dataset shows a similar cohort effect for the quantitative trait MAXDRINKS, the "maximum number of drinks ever consumed in a 24-hour period," with higher consumption levels in younger cohorts. This trait is of interest due to a prior genome screen indicating a maximal LOD of 3.5 on chromosome 4 near the alcohol dehydrogenase (ADH) gene cluster. Cohort differences for males and females born before and after 1950 are seen in the COGA control sample, the COGA probands, and in first degree relatives of COGA probands.

Using the control sample to establish quantile values for MAXDRINKS in these two cohorts for males and females separately, we obtained a cohort-corrected maximum drinks phenotype. A variance-components linkage screen for this adjusted phenotype on chromosome 4 resulted in a maximal LOD score of 4.5 also near the ADH gene cluster. A cohort-adjusted genome screen thus can refine the region of linkage and aid efforts to identify the trait-influencing gene.

Program Nr: 224 from 2002 ASHG Annual Meeting

**Natural variation in gene expression in humans.** *V.G. Cheung[1], L.K. Conlin[1], T.M. Weber[1], K-Y. Jen[1], M. Morley[1], R.S. Spielman[2].* 1) Pediatrics, Univ of PA/Children's Hospital, Philadelphia, PA; 2) Genetics, Univ of PA, Philadelphia,PA.

Variation at the DNA sequence level in the human genome is well characterized. However, the extent of variation at the level of RNA abundance among normal individuals remains largely unknown. We have examined the variation in gene expression of 5,000 genes among unrelated individuals in the CEPH families and begun to map the determinants that control this variation.

We used cDNA microarrays to find the genes whose expression levels in lymphoblastoid cell lines vary most among 35 individuals. Expression levels in each individual were measured with 4 replicates. For each gene, we calculated a variance ratio, to assess variability between individuals relative to measurement noise. A large value of this variance ratio therefore indicates high variability in expression, even after accounting for variability in replicated measurements. We then ranked the genes by this measure and selected the genes with the largest variance ratio as candidates for further analysis. We have determined the variance ratio for 5,000 genes in 35 unrelated individuals who are the parents in the CEPH Utah pedigrees. The range of variance ratios is 0.4 to 64 and the median is 2.5. For the 5% of genes with the highest variance ratios, the range of expression level among the 35 individuals is 2.4- to 17-fold.

To investigate the genetic basis of variation in gene expression, we followed up several of the highly variable genes. The transcript levels of ST3GALVI, ACTG2, GK and HNRPA2B1) were examined by a complementary technique, quantitative RT-PCR, in a total of 50 unrelated individuals, and in sibs in CEPH families and pairs of monozygotic twins. We found that the more closely related the individuals are, the smaller is the variability in expression level, thus providing evidence of familial aggregation. The results suggest that the genetic differences between individuals contribute substantially to the variation in gene expression. The sites of these genetic differences are being mapped using the marker genotypes available for the same CEPH families.

Program Nr: 225 from 2002 ASHG Annual Meeting

**A multipoint linkage disequilibrium mapping approach: incorporating evidence of linkage and linkage disequilbrium from unlinked region.** *F.C. Hsu[1], K.Y. Liang[2], T.H. Beaty[3], CSGA[4].* 1) Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 2) Biostatistics, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 3) Epidemiology, The Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; 4) CSGA, The Collaborative Study of The Genetics of Asthma (supported by NHLBI).

Gene mapping for complex diseases is still a challenge in genetic studies. For family-based studies, the method for detecting linkage and linkage disequilibrium (LD) with one locus at a time may not capture the assumed interaction between trait loci efficiently. We propose a multipoint LD approach for assessing the evidence of linkage and LD in a targeted region by incorporating evidence from the unlinked region using the case-parent trio design. The paternal and maternal preferential transmission statistics defined in Liang et al. (2001) are the primary statistics for this approach. Our generalized estimating equation (GEE) method builds on a model for the expected preferential transmission statistic from the targeted region conditional on the statistic from the unlinked region. It is valid regardless of the underlying genetic model. The major assumption is that there is no more than one trait locus in both the targeted region and unlinked region. Our goal is to estimate the map position of a trait locus and to calculate its confidence interval in the targeted region by incorporating evidence of linkage and LD from the unlinked region. Finally, we apply this approach to the chromosomes 8 and 11 data in the African American subset of the collaborative study on the genetics of asthma (CSGA). Previous analysis using the GEE approach developed by Liang et al. (2001) suggested strong evidence of linkage and LD on chromosome 11, but only marginal evidence on chromosome 8. While conditioning on marker D11S937 on chromosome 11, the trait locus on chromosome 8 is located at 11.67 cM with a 95% confidence interval of (8.75, 14.59) and the test statistic shows significant evidence of linkage and LD (chi-squared statistic = 9.86 with 3 df, p-value = 0.0198) in the targeted region.

Program Nr: 226 from 2002 ASHG Annual Meeting

**Interferon regulatory facter 6 (IRF6) is mutated in two orofacial clefting disorders, Van der Woude and Popliteal Pterygium syndromes.** *S. Kondo[1], B.C. Schutte[1], R.J. Richardson[2], B.C. Bjork[3], A.S. Knight[2], Y. Watanabe[1], E. Howard[2], M.J. Dixon[2], J.C. Murray[1].* 1) Department of Pediatrics, The University of Iowa, Iowa City, IA, USA; 2) Department of Dental Medicine and Surgery, University of Manchester, Manchester, UK; 3) Harvard University, Boston, MA, USA.

Non-syndromic cleft lip and palate (NSCLP) is one of the most common craniofacial malformations, with a prevalence of 1/500 to 1/2500 births, depending on geographic origin. The causes of orofacial clefts are complex, involving multiple genetic and environmental factors. One approach we use to identify genetic factors is to study a disorder with a closely related phenotype that exhibits a clear Mendelian pattern of inheritance. Of the more than ~150 Mendelian disorders that include orofacial clefts, we focused on Van der Woude syndrome (VWS) because it displays an autosomal dominant pattern of inheritance, it is the most frequent syndromic form of cleft lip and palate, and its only distinguishing feature is the presence of pits in the lower lip in about 70% of VWS cases. We previously mapped the VWS locus to a 350 kb region at chromosome 1q32-q41. This region contains at least 11 genes, including the IRF6 gene. IRF6 belongs to a family of transcription factors that are best known for regulating interferon expression following viral infection. DNA sequence analysis of 151 unrelated individuals with VWS identified 55 mutations in the IRF6 gene, including a stop codon in the affected sib of a monozygotic twin pair discordant for VWS. In addition, we discovered unique mutations in 13 unrelated individuals affected with Popliteal Pterygium syndrome, a similar orofacial clefting disorder that includes webbing of the lower limbs. IRF6 is highly expressed along the medial edge of the fusing palate and in skin, tooth bud and external genitalia, a pattern that is consistent with the affected tissues. We conclude that mutations in the IRF6 gene cause VWS and PPS, and that IRF6 gene is essential for orofacial, limb, and genital development.

Program Nr: 227 from 2002 ASHG Annual Meeting

**Mutation of a Tubulin-Specific Chaperone, *TBCE*, Causes the HRD/Sanjad-Sakati/Autosomal Recessive Kenny-Caffey Syndrome.** *G.A. Diaz[1], A. Al Aqeel[2,5], B.D. Gelb[1], R. Gordon[1], R. Gorodischer[3], S. Gregory[4], N. Grossman[3], E. Hershkovitz[3], M. Kambouris[5], K.T.S. Khan[6], B. Loeys[7], B.F. Meyer[5], G. Mortier[7], R. Parvari[3], N. Sakati[5], R. Sharony[8], R. Weiner[1], A. Zecic[7] and The HRD/Sanjad-Sakati/Autosomal Recessive Kenny-Caffey Syndrome Consortium.* 1) Mount Sinai School of Medicine, New York, NY; 2) Riyadh Armed Forces Hospital, Riyadh, Saudi Arabia; 3) Soroka Medical Center and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel; 4) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 5) King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; 6) Al-Jahra Hospital, Safat, Kuwait; 7) Ghent University Hospital, Ghent, Belgium; 8) The Genetic Institute, Sapir Medical Center, Meir Hospital, Sackler School of Medicine, Tel Aviv University, Israel.

The syndrome of hypoparathyroidism, mental retardation, facial dysmorphism and growth failure (HRD or Sanjad-Sakati syndrome (SSS); MIM 241410), is a pleiotropic autosomal recessive disorder. Pedigrees also featuring osteosclerosis and recurrent bacterial infections have been classified as autosomal recessive Kenny-Caffey syndrome (AR-KCS; MIM 244460). Both traits have previously been mapped to chromosome 1q43-44 and shown to share an ancestral haplotype suggesting a common founder mutation. We have refined the critical region to a ~230-kb interval and identified mutations of the *TBCE* gene in affected individuals. *TBCE* encodes one of several chaperone proteins required for the proper folding of a-tubulin subunits and the formation of a/b tubulin heterodimers. Consistent with the genetic lesion, MT density was decreased at the MT-organizing center of fibroblast and lymphoblastoid cells derived from affected subjects. Immunofluorescence and ultrastructural studies revealed striking disturbances in membrane-bounded organelles that are known to interact with MTs, such as the Golgi complex, endoplasmic reticulum, and late endosomes. These findings demonstrate that HRD/SSS/KCS is a chaperone disease caused by a defect in the tubulin assembly pathway and establishes a novel connection between tubulin physiology and parathyroid development.

Program Nr: 228 from 2002 ASHG Annual Meeting

**Gene conversion is the major cause of mutations in Shwachman-Diamond Syndrome.** *G.R.B. Boocock[1,4], J.A. Morrison[1], M. Popovic[1,4], N. Richards[1], L. Ellis[2,5], S.L. Goobie[1,4], P.R. Durie[2,3,5], J.M. Rommens[1,4].* 1) Program in Genetics and Genomic Biology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; 2) Program in Integrative Biology, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Division of Gastroenterology and Nutrition, The Hospital for Sick Children, Toronto, Ontario, Canada; 4) Department of Molecular and Medical Genetics, The University of Toronto, Toronto, Ontario, Canada; 5) Department of Pediatrics, The University of Toronto, Toronto, Ontario, Canada.

Shwachman-Diamond syndrome (SDS [MIM 260400]) is an autosomal recessive disorder with clinical features including exocrine pancreatic insufficiency, hematological dysfunction, and skeletal abnormalities. Mutations have been identified in an uncharacterized gene residing within the 1.9cM disease interval at 7q11 delineated by linkage and haplotype analysis in family studies. The gene, with a 1.6kb transcript, encodes a predicted protein of 250 amino acids. A second copy, with 97% nucleotide sequence identity, resides within a locally duplicated genomic block of at least 88kb, and appears to be a pseudogene. Reoccurring mutations, the apparent result of recombination between the duplicated gene copies, were found in 90% of unrelated SDS patients (n=157), with 59% carrying two converted alleles. The extent of the converted segments varied but consistently included at least one of two critical sequence changes predicted to result in truncation of the encoded protein. Other disease alleles involve missense mutations and insertion/deletion changes distinct from those in the pseudogene. The gene is a member of a highly conserved protein family of unknown function, with putative orthologs in diverse species ranging from archaebacteria to eukaryotes. The archaeal orthologs are located in highly conserved operons that include homologs of subunits of the eukaryotic exosome, suggesting that SDS may be the result of a cellular defect in an aspect of RNA metabolism that is essential for the development of the exocrine pancreas, hematopoiesis and chrondrogenesis.

Program Nr: 229 from 2002 ASHG Annual Meeting

**Identification of the gene most commonly involved in Bardet-Biedl syndome (BBS1) and evaluation of triallelic inheritance involving the *BBS1* gene.** *K. Mykytyn[1], D.Y. Nishimura[1], C. Searby[1], H. Yen[1], A.S. Cornier[2], G.F. Cox[3], R. Carmi[4], S.C. Chandrasekharappa[5], F.S. Collins[5], E.M. Stone[6], V.C. Sheffield[1]*. 1) Dept of Pediatrics, Univ Iowa, Iowa City, IA; 2) Dept of Biochemistry, Ponce School of Medicine, Ponce, Puerto Rico; 3) Childrens Hospital, Boston, MA; 4) Genetics Institute, Soroka Medical Center, Beer-Sheva, Israel; 5) NHGRI, NIH, Bethesda, MD; 6) Dept of Ophthalmology, Univ Iowa, Iowa City, IA.

Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder with the primary features of obesity, pigmentary retinopathy, polydactyly, renal malformations, mental retardation, and hypogenitalism. BBS has been linked to six loci and the *BBS2*, *BBS4*, and *BBS6* (*MKKS*) genes have recently been identified. The BBS1 locus was initially mapped to a 26 cM interval on chromosome 11. We used haplotype analysis of several extended families to narrow the disease interval between markers D11S913 and AFMa190yd5. One gene in this region was of interest because the predicted protein sequence showed modest similarity to BBS2. Sequencing of this gene, now known as *BBS1*, revealed a number of mutations, including nonsense, missense, splice site and frameshift mutations. One of the mutations, a non-conservative methionine to arginine substitution at codon 390, was found in 22 out of 60 unrelated North American BBS probands. Fifteen of these individuals were homozygous for the variant. Our results demonstrate that mutations in the *BBS1* gene account for about half of BBS cases and M390R is a common *BBS1* mutation in the North American population. Recently, it has been suggested that three mutant alleles (two at one locus, and a third at a second locus) may be required for manifestation of BBS (triallelic inheritance). The identification of the *BBS1* gene provides the opportunity to evaluate this hypothesis. Sequencing of the other known BBS genes in 30 individuals who possess two *BBS1* mutations did not reveal any additional mutations. Additionally, sequencing of *BBS1* in BBS2, BBS4, and BBS6 patients did not reveal a third mutant allele. These data indicate that triallelic inheritance of BBS is not common.

Program Nr: 230 from 2002 ASHG Annual Meeting

**Mutation of CRELD1 associated with an atrioventricular canal defect.** *C.L. Maslen[1], S.W. Robinson[1], C.D. Morris[2]*. 1) Dept Medicine and Molec/Med Genetics; 2) Medical Informatics and Outcomes Research, Oregon Health & Science Univ, Portland, OR.

CRELD1 is a novel membrane bound glycoprotein with characteristics of a cell adhesion molecule. The CRELD1 gene maps to chromosome 3p25.3 in the vicinity of the AVSD2 locus. Expression in the myocardium and AV canal endocardial cushions suggests a role in valvuloseptal morphogenesis. We have evaluated CRELD1 as a candidate gene for isolated AV canal defects. Subjects with isolated complete or partial AVSD were recruited through the Oregon Congenital Heart Defects Registry. Genomic DNA was collected and analyzed for changes in CRELD1. One subject with an ostium primum ASD had a heterozygous C to T transition in exon 9 (C4201T). This alteration was not detected in 400 normal chromosomes, indicating that it is a mutation rather than a polymorphism. However, the mutation was also detected in the apparently unaffected father, brother and sister of the proband. This suggests that there may be incomplete penetrance of the trait, or that there is variable expression of disease severity with subclinical defects going undetected in some mutation carriers. The C4201T mutation results in a substitution of cysteine for arginine at amino acid 329 (R329C) in a calcium binding EGF domain. 3D modeling of the domain indicates that the introduced free cysteine will be directed outward available for disulfide bond formation. Analysis of expressed mutant protein showed that it forms abnormal aggregates, which is likely to result in intracellular retention of the protein. Based on these data we propose that this is a pathogenic mutation and that CRELD1 is the AVSD2 gene.

Program Nr: 231 from 2002 ASHG Annual Meeting

**GRACILE syndrome is caused by a point mutation in *BCS1L*, suggesting a new role of the BCS1L in iron metabolism.** *I. Visapaa[1,2], V. Fellman[3], J. Vesa[1], A. Dasvarma[4], J.L. Hutton[5], V. Kumar[6], G.S. Payne[5], M. Makarow[6], R. Van Coster[7], R.W. Taylor[8], D.M. Turnbull[8], A. Suomalainen[9], L. Peltonen[1,2]*. 1) Dept. of Human Genetics, UCLA School of Medicine, Los Angeles, USA; 2) Dept. of Molecular Medicine, National Public Health Institute, and Dept. of Medical Genetics, University of Helsinki, Finland; 3) Hospital for Children and Adolescents, Helsinki University Central Hospital, Finland; 4) Murdoch Childrens Research Institute, Melbourne, Australia; 5) Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, USA; 6) Institute of Biotechnology, University of Helsinki, Finland; 7) Dept. of Pediatrics, Ghent University Hospital, Belgium; 8) Dept. of Neurology, University of Newcastle upon Tyne, UK; 9) Dept. of Neurology and Programme of Neurosciences, University of Helsinki, Finland.

GRACILE syndrome (MIM 603358) is a recessively inherited lethal disease characterized by fetal growth retardation, lactic acidosis, aminoaciduria, cholestasis and abnormalities in iron metabolism. We have earlier localized the causative gene to a 1.5 cM region on chromosome 2q33-37. Here we report the molecular defect causing this metabolic disorder by identifying a homozygous missense mutation, resulting in an S78G amino acid change, in the *BCS1L* gene in Finnish GRACILE patients, as well as five different mutations in three British infants. BCS1L, a mitochondrial inner membrane protein, is a chaperone necessary for the assembly of mitochondrial respiratory chain complex III. Pulse-chase experiments performed in COS-1 cells indicated that the S78G amino acid change results in instability of the polypeptide, and yeast complementation studies revealed a functional defect in the mutated BCS1L protein. Four different mutations in the *BCS1L* gene have been reported earlier in Turkish patients with a distinctly different phenotype (de Lonlay et al. 2001). Interestingly, the British and Turkish patients had complex III deficiency, whereas normal activity was found in the Finnish GRACILE patients, implying that BCS1L has another, non-characterized but essential cellular function that is putatively involved in iron metabolism.

Program Nr: 232 from 2002 ASHG Annual Meeting

**Homozygosity for a missense mutation in fibulin-5 (FBLN5) results in a severe form of cutis laxa.** *B. Loeys[1,5], L. Van Maldergem[2], G. Mortier[1], P. Coucke[1], S. Gerniers[3], J.M. Naeyaert[4], A. De Paepe[1].* 1) Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; 2) Centre de Genetique Humaine, Institut de Pathologie et de Genetique, Loverval, Belgium; 3) Department of Pediatrics, Sint-Lucas Hospital, Ghent, Belgium; 4) Department of Dermatology, Ghent University Hospital, Ghent, Belgium; 5) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, USA.

Hereditary cutis laxa comprises a heterogeneous group of connective tissue disorders characterized by loose skin and variable systemic involvement. Autosomal dominant and recessive as well as X-linked forms have been described. Some dominant forms are caused by mutations in the elastin-gene (ELN). The X-linked form is now classified in the group of copper transport diseases. The genetic defect underlying the autosomal recessive (AR) forms of cutis laxa is not known. The phenotypic abnormalities recently observed in a fibulin-5 knock-out mouse model are reminiscent of human AR cutis laxa type I. Both share cutis laxa, lung emphysema and arterial involvement. We describe a large consanguineous Turkish family with four affected members. The proband presented with cutis laxa, severe emphysema and supravalvular aortic stenosis. Histologic examination of skin sections stained with van Gieson showed poorly developed elastin fibers with a marked gross-granular appearance. Three other children in another branch of the family presented with the same cutis laxa phenotype. Molecular study of the fibulin-5 (FBLN5) gene in this family demonstrated the presence of a homozygous missense mutation (T998C) in the FBLN5 gene resulting in a serine to proline (S227P) substitution in the fourth calcium binding EGF-like domain of the fibulin-5 protein. This serine-residue is highly conserved within this domain across species and in other human fibulins. This amino acid substitution (S227P) is predicted to have important structural and functional consequences for normal elastogenesis. As such, we provide the first evidence that a genetic defect in fibulin-5 (FBLN5, also known as EVEC or DANCE) is responsible for an autosomal recessive form of cutis laxa in humans.

Program Nr: 233 from 2002 ASHG Annual Meeting

**Ferritin crystals are cataracts in hereditary hyperferritinemia cataract syndrome: lessons from an extreme protein deposition disease.** *D.G. Brooks[1], T. Baradet[2], I. Devaux[3], C. Beaumont[3], D. Stambolian[2]*. 1) Div Med Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Depts Ophthalmology & Genetics, Univ Pennsylvania, Philadelphia, PA; 3) INSERM, Paris France.

Loss of protein solubility is an important pathogenetic mechanism in multiple diseases. The extreme of protein insolubility is crystallization, a rare fate for native proteins in vivo. A striking example of in vivo protein crystallization is the human disease hereditary hyperferritinemia cataract syndrome (HHCS). HHCS is defined by autosomal dominant inheritance of cataracts and elevated ferritin levels. HHCS results from specific mutations of the L ferritin gene that disrupt the iron responsive element, a cis-acting inhibitor of L ferritin mRNA translation. This leads to L-ferritin over expression and accumulation as a 440 kD spherical 24-mer capable of storing iron. In human lens, excess ferritin forms crystalline cataracts that diffract light causing patients to suffer progressive glare/vision impairment. To understand the natural history of this rare example of in vivo protein crystallization, a transgenic mouse model was developed. A typical HHCS mutation was engineered into the normal mouse L ferritin gene and the transgene was injected into fertilized oocytes. Three lines of mice were established that over express mouse L ferritin up to 10 fold in all tissues examined. Extracellular ferritin crystals are observed in mouse lens as well as in bone marrow. Large ferritin inclusions are observed in the cytoplasm and nucleus of many tissues. In liver, pancreas and heart there is significant stainable iron associated with these ferritin inclusions. Chemical measurement of the non heme iron in these transgenic mice shows a 1.5-2 fold increase in liver and spleen as compared to control littermates. This transgenic mouse model recapitulates the HHCS phenotype as well as highlighting a sequence of events in protein deposition: protein over-expression/accumulation, aggregation into inclusions, organization into paracrystalline deposits and crystallization. Crystals have only been observed outside cells to date suggesting that intracellular environments may inhibit ferritin crystallization.

Program Nr: 234 from 2002 ASHG Annual Meeting

**The Fanconi Anemia/BRCA pathway is not involved in the recognition and initial processing of DNA interstrand crosslinks (ICL).** *A. Rothfuss, M. Grompe.* Molecular and Medical Genetics, OHSU, Portland, OR.

DNA interstrand crosslinks (ICL) prevent separation of DNA strands therefore blocking essential cellular processes such as replication, transcription and segregation. The importance of elucidating ICL repair pathways is further strengthened given the hypersensitivity of Fanconi Anemia (FA) and BRCA1/2 mutant cells to ICL-inducing agents. Currently, the mechanisms of ICL repair in mammals are largely unknown. We have previously shown that ICL-treated cells arrest in late S-phase regardless of when the damage was introduced during the cell cycle (Akkari et al. (2000), MCB, 20; 8283-8289). FA mutant cells displayed a significantly prolonged S-phase arrest suggesting that the FA pathway plays a role in S-phase repair of ICL. This model was strengthened by the recent discovery that BRCA2, known to control HR during S-phase, is a FA gene. We therefore wished to determine whether the FA proteins function to detect ICL during S-phase. For this purpose we developed a modified protocol of the alkaline single cell gel electrophoresis (comet assay). This method was able to detect PUVA-induced ICL with a ten-fold higher sensitivity compared to previous methods, giving a detection limit of ~600 ICL/genome. We found that unsynchronized primary wild-type fibroblasts incise ICL very efficiently, leading to a complete unhooking of low doses of ICL after 2h. Within 24h, even high amounts of ICL are completely incised. Furthermore, cells synchronized in the G1 phase of the cell cycle also showed efficient and rapid ICL uncoupling. This was also true for FA cells, therefore suggesting that the role of the FA pathway in ICL repair is not in the initial recognition of these lesions. Immunocytological studies at different time points after ICL induction showed positive staining for gH2AX, suggesting that double-strand breaks (DSB) occur during ICL repair in wild-type cells. These results suggest a modified model in which ICL are recognized and incised very rapidly, independent of S-phase and independent of FA proteins. DSB occur during repair, possibly during S-phase and we are currently testing the hypothesis whether the FA pathway plays a role in the generation of DSB.

Program Nr: 235 from 2002 ASHG Annual Meeting

**Lack of Fanconi anemia DNA damage-induced signalling results in a DNA double-strand break repair defect.**
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Fanconi anemia (FA) is a heterogeneous autosomal recessive disease characterized by congenital abnormalities, bone marrow failure, and an increased incidence of cancers. Cells cultured from FA patients display elevated spontaneous chromosomal breaks and deletions, and are hypersensitive to bifunctional alkylating agents. For this reason, it has been hypothesized that FA cells are deficient in DNA repair. We found that intact human FA fibroblasts from complementation groups A, C, D, and G rejoined linearized plasmids with an efficiency of ~4%, while non-FA fibroblasts were capable of rejoining the same plasmids with ~28% efficiency. Retrovirus-mediated expression of the respective FA cDNAs in these cells restored their end-joining efficiency to that of normal cells. FA fibroblasts were also significantly more sensitive to chromosomal DNA double-strand breaks induced by restriction enzyme than were their retrovirally-corrected counterparts. Electroporation of 20 units of the restriction enzyme PvuII caused ~56% cell death in human FA fibroblasts from complementation groups A, C, D, and G. In contrast, similar treatment of multiple non-FA and retrovirally-corrected FA fibroblasts resulted in only ~4% cell death. Both mouse and hamster FA model cells displayed a similar hypersensitivity to RE-induced cell death. These data indicate that FA fibroblasts have deficient DNA double-strand break repair. Although DNA double-strand breaks can be repaired via both homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways, these data along with other recently generated data suggest that FA fibroblasts are deficient in an NHEJ pathway that is independent from Ku-mediated NHEJ. Furthermore, we found that extrachromosomal HR is elevated, not reduced, in human FA fibroblasts. These data support the hypothesis that FA proteins participate in a DNA damage-induced signal-transduction cascade. Failure to activate this response in FA cells results in a dramatic defect in a novel cellular DNA double-strand break repair activity. A defect in double-strand break repair could provide an attractive explanation for much of the pathology associated with FA.

Program Nr: 236 from 2002 ASHG Annual Meeting

**The current contribution of chromosomal anomalies to the occurrence of congenital heart defects: findings from population-based study.** *L.D. Botto[1], R.M. Campbell[2], S.A. Rasmussen[1], A. Correa[1], L. O'Leary[1], K. Coleman[2], K. May[3], P.M. Fernhoff[3]*. 1) Birth Def & Gen Dis, NCEH, Ctrs Disease Control & Prevent, Atlanta, GA; 2) Sibley Heart Center, Childrens Healthcare of Atlanta, Atlanta, GA; 3) Department of Medical Genetics, Emory University, Atlanta, GA.

The contribution of chromosomal anomalies to congenital heart defects has long been appreciated. However, few population-based data are available, particularly for recent times when new tests are available for conditions such as the 22q11.2 deletion. Our aim was to evaluate the frequency and distribution of chromosomal anomalies in a large, recent birth cohort to assess the contribution of such anomalies to the occurrence of congenital heart anomalies in a well-defined population. We evaluated data on babies born from 1994 through 1999 to mothers who resided in the metropolitan Atlanta area. We used data from the Metropolitan Atlanta Congenital Defects Program (MACDP) and the Division of Medical Genetics at Emory University. MACDP is a population-based birth defects registry that uses active case-ascertainment from multiple sources to identify babies with birth defects. We identified 2,396 babies with congenital heart defects among 255,849 resident births, for a population-based prevalence of heart defects of 0.9 percent. Of these, 320 (13.4%) had a diagnosis of a chromosomal anomaly. Of this group, trisomy 21, 22q11.2 deletion, and trisomy 18 were the most common chromosomal diagnoses (58.1, 10.9, and 10.3% respectively, and 79.4% in total). Numerous other conditions (supernumerary chromosomes, deletions, rearrangements) were identified among the remainder, but for most their contribution to the overall occurrence of heart defects was small (2% or less). We conclude that, currently, chromosomal anomalies contribute to at least 1 in 7 cases of heart defects. Whereas trisomy 21 still accounts for most cases, the 22q11.2 deletion has emerged as the second largest contributor to heart defects, and because of its better survival compared to other chromosomal anomalies (eg, trisomies 13 and 18), it may account for an increasing proportion of cases among older children and adults.

Program Nr: 237 from 2002 ASHG Annual Meeting

**Molecular etiology of vascular anomalies:The role of Jagged1 and the Notch Signaling Pathway.** *B.M. Kamath[1], N.B. Spinner[1], K.M. Emerick[2], C. Booth[3], A.E. Chudley[4], L. Bason[1], D.A. Piccoli[1], I.D. Krantz[1]*. 1) Children's Hospital of Philadelphia & University of Pennsylvania Medical School, Philadelphia, PA; 2) Northwestern University Medical School,Children's Memorial Hospital, Chicago, IL; 3) Lutheran General Children's Hospital, Park Ridge, IL; 4) Children's Hospital & University of Manitoba, Winnipeg, Manitoba.

Vascular anomalies are a significant cause of morbidity and mortality yet often the etiology is unknown. The existence of familial cases suggests a genetic component. Alagille syndrome (AGS,OMIM#118450) is a dominantly inherited disorder caused by mutations in Jagged1 (JAG1), a ligand in the Notch signaling pathway (NSP). The main manifestations of AGS are cholestasis,cardiac defects primarily involving the pulmonary arteries,butterfly vertebrae,posterior embryotoxon and facial dysmorphism. Intracranial hemorrhages are seen in 16% of patients. Isolated reports of intracranial and peripheral vessel anomalies also exist. Studies suggest that a vascular developmental defect may underlie the variable AGS phenotype. Evidence supporting a role for JAG1/NSP in vasculogenesis includes 1)expression of NSP members in vascular endothelium 2)JAG1 mutant homozygote mice die from hemorrhage during embryogenesis due to defects in angiogenic remodeling 3)Notch1 mutant and Notch1/Notch4 mutant mice display similar defects and 4)CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy) is caused by Notch3 mutations. Review of our AGS database of 250 individuals identified 13 cases (5%) with vascular involvement other than pulmonary artery anomalies. Two had basilar artery aneurysms and one had an internal carotid artery aneurysm. Six further patients had intracranial bleeds without documented vessel abnormalities. Moyamoya disease was described in 1 patient. Three of the 13 cases had aortic aneurysms.The vascular anomalies described in this cohort identify a potentially devastating complication of AGS. We have reviewed the evidence implicating JAG1 and the NSP in vascular development and suggest that vasculopathy is the primary abnormality in AGS and implicates JAG1 and the NSP in the etiology of vascular anomalies.

Program Nr: 238 from 2002 ASHG Annual Meeting

**Echocardiographic Evaluation of Parental and Sibling Risk Associated with Pediatric Left Ventricular Outflow Tract Lesions.** *K.L. McBride[1], M.B. Lewin[2], R. Pignatelli[3], S.D. Fernbach[3], A. Combes[3], W. Lam[4], L. Bezold[3], T. Ho[1], W. Craigen[3], N. Kaplan[5], J.A. Towbin[3], J.W. Belmont[1]*. 1) Dept Human & Molecular Gen, Baylor Col Medicine, Houston, TX; 2) Division of Pediatric Cardiology University of Washington, Seattle, WA; 3) Division of Pediatric Cardiology, Baylor College of Medicine, Houston, TX; 4) Department of Pediatrics, University of California, San Francisco, San Francisco, CA; 5) National Institutes of Health, National Institute of Environmental Sciences, Bethesda, MD.

OBJECTIVES: This study assessed the occurrence of unsuspected cardiac anomalies in first-degree relatives of children with left ventricular outflow tract obstructive (LVOTO) malformations. METHODS: 67 pediatric patients with a non-syndromic LVOTO malformation were enrolled, including aortic valve stenosis (AVS; n=14), coarctation of the aorta (CoA; n=36), hypoplastic left heart syndrome (HLHS; n=17), and Shone syndrome (n=3). Echocardiograms were performed on 164 first-degree relatives of these patients. RESULTS: 36 cardiac defects were found in 15 of 62 mothers, 10 of 61 fathers, 8 of 20 brothers and 3 of 18 sisters. 3 studies, all on mothers, were excluded from analysis due lupus (1), pregnancy (1), and poor study (1). Left heart lesions included mitral valve thickening, redundant chordae tendinae, interventricular septum thickening, bicuspid aortic valve (BAV), aortic valve thickening, and ascending aorta dilation. The risk of finding a defect was not different between families with more severely vs. less severely affected probands, nor between families with multiple vs. single probands. The overall risk of identifying a congenital cardiac abnormality in a first degree relative was 22.4%. The most common single defect was BAV (n = 9) at 5.6 %. The relative risk of BAV is 6.2. CONCLUSIONS: The presence of a congenital left heart lesion greatly increases the risk of identifying cardiac abnormalities in a parent or sibling. These results are supportive of a complex genetic etiology for left heart obstructive lesions. The parents and siblings of affected patients should be screened by echocardiography and long-term medical follow-up may be required for a significant minority.

Program Nr: 239 from 2002 ASHG Annual Meeting

**Linkage of Familial Bicuspid Aortic Valve with Aortic Aneurysm to Chromosome 15q.** *D.L. Goh[1,2], L.F Han[1], D.P. Judge[1], J.A. Geutbner[1], I. McIntosh[1], A. Patel[3], G.H. Thomas[3], C.T. Basson[4], D.M. Milewicz[5], H.C. Dietz[1,6].* 1) Johns Hopkins University School of Medicine, Baltimore, MD; 2) National University of Singapore, Singapore; 3) Kennedy Krieger Institute, Baltimore, MD; 4) Weill Medical College of Cornell University, New York, NY; 5) University of Texas-Houston Medical School, Houston, TX; 6) Howard Hughes Medical Institute.

Bicuspid aortic valve (BAV) is the most common congenital heart malformation, with an estimated incidence of 1%. BAV can be associated with ascending aortic aneurysm (AscAA), historically attributed to hemodynamic perturbation caused by valve malformation. Comprehensive evaluation of multiple pedigrees segregating BAV with AscAA revealed a high incidence of individuals with AscAA alone, suggesting that BAV and AscAA are both primary manifestations of a single gene defect with variable expression. BAV/AscAA segregates as an autosomal dominant trait with incomplete penetrance and wide variation in the age of onset of aortic enlargement, ranging from infancy to mid-adult life. Maximal enlargement of the aorta frequently occurs above the sinotubular junction, distinguishing BAV/AscAA from Marfan syndrome or previously reported forms of familial AscAA. We identified a patient with multiple congenital anomalies that included BAV, AscAA, sensorineural hearing loss, growth failure, developmental delay and dysmorphic facies. This was associated with a *de novo*interstitial deletion [46,XX,del(15)(q25q26.1)] spanning 7Mb, as mapped by FISH and loss of heterozygosity. This suggested that a gene for BAV/AscAA maps within this critical interval. Ten multiplex families segregating BAV/AscAA were genotyped with polymorphic markers spanning the deleted interval and nine were consistent with linkage to this region of chromosome 15q with a maximum single-point LOD score of 3.60. These data mandate periodic echocardiographic follow-up of all first-degree relatives of individuals with BAV/AscAA including those without valve malformation. Further refinement of this locus and identification of the gene(s) responsible for BAV/AscAA will allow molecular assignment of risk and provide important insight regarding the molecular determinants of aortic wall homeostasis.

Program Nr: 240 from 2002 ASHG Annual Meeting

**Molecular and cytogenetic evidence that cardiofaciocutaneous (CFC) syndrome is distinct from Noonan syndrome (NS)and that it's locus is not in the chromosome region 12q21.2q22.** *M.I. Kavamura[1,2], M. Zollino[1], M.G. Pomponi[1], R. Lecce[1], M. Murdolo[1], D. Brunoni[2], J.M. Opitz[1,3], G. Neri[1]*. 1) Istituto di Genetica Medica, Università Cattolica del Sacro Cuore, Rome, Italy; 2) Centro de Genética Médica, Universidade Federal de São Paulo - Escola Paulista de Medicina, São Paulo, Brazil; 3) University of Utah, Salt Lake City, Utah, USA.

Cardiofaciocutaneous (CFC) syndrome, described in 1986 by Reynolds et al., is a multiple congenital anomalies/mental retardation syndrome characterized by congenital heart defects, characteristic facial appearance, short stature, ectodermal abnormalities, and mental retardation. All reported cases are sporadic, have apparent normal chromosomes, and were born to non-consanguineous parents. CFC syndrome's main differential diagnosis is made with Noonan syndrome (NS). CFC and NS share similar cardiac defects, craniofacial appearance and growth retardation. However, other clinical aspects such as degree of mental impairment, speech delay, ectodermal abnormalities, and severity of failure to thrive, can distinguish the two conditions. Two patients with a CFC-like phenotype and the same deletion on 12(12q21.2q22)have been reported [Rauen et al., 2000 and 2002]. The CFC syndrome diagnosis was proposed for both patients, although they were not typical cases. In order to verify the presence of microdeletions within this area in typical CFC patients, we performed FISH analysis with 12 BAC probes in 17 typical CFC patients. No microdeletions were found. The findind of missense mutations of the *PTPN11* gene in 45-50%; of NS patients, with penetrance of almost 100%; [Tartaglia et al. 2001 and 2002], strongly suggests that mutations of the *PTPN11* are one of the causes of NS. We have sequenced the entire coding-region of the *PTPN11* as well as its intron boundary regions, and studied its cDNA in 10 typical cases of the CFC syndrome. No base changes or deletions were detected. Based on the above findings we conclude: a) that the region 12q21.2q22 is not a candidate region for the CFC syndrome; b) that *PTPN11* mutations or deletions are not found in CFC patients, supporting the contention that CFC and Noonan syndromes are distinct genetic entities.

Program Nr: 241 from 2002 ASHG Annual Meeting

**Dosage sensitive role of Tbx1 in the etiology of VCFS/DGS in mouse models.** *J. Liao, J.S. Arnold, S. Nowotschin, S. Lipner, R.K. Pandita, Z. Li, A. Skoultchi, B.E. Morrow.* Albert Einstein College of Med, Bronx, NY.

Velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS) is associated with hemizygous deletions on 22q11 and is characterized by craniofacial anomalies, thymus hypoplasia and cardiovascular defects. Recently, Tbx1, a gene hemizygously deleted on 22q11 was found to be a strong candidate for the syndrome in mouse models. While heterozygotes survived in normal mendelian ratios and had mild cardiovascular defects, homozygotes died in the perinatal period with major malformations of the derivatives of the pharyngeal arches and the ear. The Tbx1 gene is a member of the T-box containing family of transcription factors that are sensitive to altered dosage. Both increased and decreased dosage of T-box family members is associated with congenital anomalies. Bacterial artificial chromosome (BAC) transgenic mice overexpresing human TBX1 and three other transgenes (PNUTL1, GP1BB and WDR14) had reduced viability, chronic otitis media, mild ear malformations, cleft palate, thymus gland hypoplasia and outflow tract heart defects. These defects were strikingly similar to those in VCFS/DGS patients and were more extensive than those observed in the Tbx1 heterozygous mice. To determine whether TBX1 or the three other human transgenes might be responsible for the defects in the BAC transgenic mice, we crossed them into a Tbx1 null background. We found that genetic complementation occurred demonstrating that overexpression of TBX1 was responsible for the VCFS/DGS phenotype in the transgenic mice. Therefore, altered TBX1 dosage may be responsible for these anomalies in the VCFS/DGS patients. As in the human condition, the penetrance in the transgenic mice was complete but the expressivity was variable. Some mice had only mild defects while others died in the perinatal period. The BAC transgenic mice were in a pure genetic background. This suggests that variable expressivity might be caused by stochastic events resulting from altered dosage and not by genetic modifiers. This has significant implications for understanding the basis for varied expressivity in human patients with VCFS/DGS.

Program Nr: 242 from 2002 ASHG Annual Meeting

**Autosomal recessive spondylocostal dysostosis: three new pathogenic alleles in *DLL3* gene.** *L. Bonafe, C. Giunta, M. Gassner, B. Steinmann, A. Superti-Furga.* Div. Metabolism and Mol. Ped., Univ. Children's Hosp., Zurich, Switzerland.

Several years ago, a cluster of individuals affected by Spondylcostal Dysostosis (SD; MIM 277300) was observed in a village from eastern Switzerland (Gassner M, Schweiz Med Wochenschr, 1982;112:791-797). We tested the hypothesis that the molecular basis for this cluster was a mutation in the *DLL3* gene, recently linked to SD (Bulman et al, Nature Genet, 2000;24:438-441). Linkage analysis around the *DLL3* locus was compatible with homozygosity by descent in only 4 of 6 affected individuals. Sequence analysis revealed 3 unreported *DLL3* mutations: four probands were homozygous for a duplication of 17 bp in exon 8 (dup1285-1301) and one individual was compound heterozygous for this duplication and for a single-nucleotide deletion in exon 5 (delC615), both mutations leading to a frameshift and premature stop codon. A further affected individual was compound heterozygous for the exon 8 duplication as well as for a R238X nonsense mutation in exon 6.

Conclusions: (1) we confirm the presence of mutations in the *DLL3* gene in patients with autosomal recessive spondylocostal dysostosis; (2) similarly to the previously reported mutations, pathogenic changes are located in the DSL domain and in the EGF repeats of the protein; (3) contrary to our initial assumption of a single allele segregating in this small community because of a founder effect, three different pathogenic alleles were observed. This may indicate that *DLL3* mutations may be not exceedingly rare.

Program Nr: 243 from 2002 ASHG Annual Meeting

**Pseudo-dominant inheritance of spondylocostal dysostosis caused by two familial *Delta-like 3* mutations.** *N.V. Whittock[1], J. Duncan[1], S. Ellard[1], P.D. Turnpenny[1,2]*. 1) Molecular Genetics Centre, University of Exeter, Exeter, UK; 2) Clinical Genetics Department, Royal Devon & Exeter Hospital, Barrack Road, Exeter EX2 5DW, UK.

The spondylocostal dysostoses (SCDs) are a heterogeneous group of disorders with severe axial skeletal malformation, characterised by multiple vertebral segmentation defects (MVSDs) and rib anomalies. Sporadic cases with diverse phenotypes are relatively common whilst monogenic SCD families have been reported with autosomal recessive (AR) and, more rarely, autosomal dominant (AD) inheritance. We have previously shown that mutations in the somitogenesis gene, *Delta-like 3 (DLL3)*, which encodes a ligand for the Notch signaling pathway, cause autosomal recessive AR SCD (Bulman et al. 2000), with a consistent pattern of abnormal segmentation. We have studied a family with SCD previously reported to show AD inheritance, in whom the phenotype is very similar to AR cases. Direct *DLL3* sequencing of the affected members of two generations demonstrated that the affected father is homozygous for a novel frameshift mutation, 1440delG, predicted to result in a novel 68 amino acid carboxy terminal peptide and a premature termination at codon 547. His two affected children are compound heterozygotes for this frameshift mutation and a novel missense mutation, G504D. Their two unaffected siblings are heterozygous for the 1440delG mutation only. The missense mutation results in the substitution of a charged polar amino acid (aspartate) for the non-polar glycine. This is the first putative missense mutation reported in the transmembrane domain of *DLL3*. The findings in this family raise the question as to whether there are any truly AD SCD families where the phenotype is MVSDs affecting the entire spine.

Program Nr: 244 from 2002 ASHG Annual Meeting

**Identification of a *Col2A1* mutation in a Micronesian family with autosomal dominant precocious osteoarthritis.**

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We describe a large Micronesian family with an autosomal dominant form of precocious osteoarthritis. In this kindred symptoms of osteoarthritis were observed as early as 2.5 years of age. The spine, major joints (particularly the hips and knees), and small joints of the hand and feet were all involved. By adulthood, osteophyte formation, disc irregularity, and platyspondyly were observed in the affected family members. To identify the causative mutation, a whole genome scan utilizing 24 family members (affected and unaffected) was performed. A marker on chromosome 12 showed strong evidence of linkage with a Lod score of 4.03 at Theta = 0. This marker maps near the *Col2A1* gene. Mutations in *Col2A1*, including single base changes, deletions, duplications and splicing defects have been observed in many inherited forms of osteoarthritis and chondrodysplasia. Sequencing of affected individuals in our family identified a C to T substitution in exon 11 of the *Col2A1* gene that cosegregated with the disease phenotype. This mutation results in an arginine to cysteine substitution at amino acid number 75 of the major triple helical domain. This mutation has previously been described in three other kindreds from the Chiloe Islands, Utah, and France all exhibiting precocious osteoarthritis. Our family represents the fourth independent report of the Arg75Cys mutation in the *Col2A1* gene in an osteoarthritis kindred. The mutation occurs at a CpG dinucleotide which may explain its independent occurrence in different ethnic groups.

Program Nr: 245 from 2002 ASHG Annual Meeting

**Living with achondroplasia: quality of life evaluation following cervico-medullary decompression.** *N.C. Ho[1,2], L.J. Brant[3], S.S. Park[1], M. North[2], M.J. Guarnieri[2], C.A. Francomano[1], B.S. Carson[2].* 1) Section of Human Genetics and Integrative Medicine, LG, NIA, NIH, Baltimore, MD; 2) Department of Pediatrics, Johns Hopkins Medical Institutions, Baltimore, MD; 3) Statistical Methods and Experimental Designs Section, RRB, NIA, NIH, Baltimore, MD.

Achondroplasia is the most common of the heritable skeletal dysplasias. Because of the potentially lethal nature of sub-occipital compression in achondroplasia, cervico-medullary decompression (CMD) at the foramen magnum is often employed to relieve the pressure on the emerging cervical cord. In this study, we assessed 109 individuals followed up longitudinally, of whom 52 (48%) patients had undergone CMD and the remaining 57 (52%) did not. 62 (57%) were males and 47 (43%) were females. 100 (92%) of the patients were Caucasians, 6 were African Americans, 1 Hispanic, and 2 of unknown ethnic origins. There were 58% between the ages of 6 and 20 years, and 42% were 21 years and older.

We used a modified SF-36 questionaire, surveying 8 health concepts: 1) physical functioning, 2) social functioning, 3) bodily pain, 4) role limitations because of physical problems, 5) role limitations because of emotional problems, 6) general mental health, 7) vitality, and 8) general health perceptions. Information was collected via telephone or mail. When asked to rate their current health, in the CMD group, 39% rated their health as excellent, 48% as good and 13% poor. In the non-CMD group, 19% claimed excellent health, 62% good health, and 19% poor, thus demonstrating a statistically significant linear trend in proportions ($c^2$ test, $p<0.05$). When asked to score their present health in comparison to their health a year ago, 33% in the CMD group claimed excellent health, 52% good, and 15% poor. In the non-CMD group, 14% rated their health excellent, 74% good, and 12% poor. The $c^2$ test of association is significant ($p<0.05$). Analyses of data pertinent to the other 7 health concepts did not show evidence of a trend or association of statistical significance. These findings suggest that cervico-medulllary decompression in achondroplasia is associated with a positive perception of general health in the long term.

Program Nr: 246 from 2002 ASHG Annual Meeting

**Genetic mapping of a canine hereditary renal cancer syndrome.** *K.E. Comstock[1], L. Moe[2], E. Kirkness[3], C. Hitte[4], L. Sabacan[1], R. Heggebo[2], E. Smith[1], T. Jonasdottir[2], F. Galibert[4], F. Lingaas[2], E.A. Ostrander[1].* 1) Fred Hutchinson Cancer Research Center, Seattle WA; 2) Norwegian School of Veterinary Science, Oslo Norway; 3) The Institute for Genome Research, Rockville MD; 4) Universite de Renne, Renne France.

We and others have previously proposed that mapping cancer susceptibility genes in the domestic dog can circumvent many difficulties associated with mapping these genes in humans. We herein describe our progress toward the localization of a gene for a canine kidney cancer syndrome called Hereditary Multifocal Renal Cystadenocarcinoma and Nodular Dermatofibrosis (RCND). RCND is a naturally occurring syndrome characterized by bilateral, multifocal tumors in kidneys, uterine leiomyomas and nodules in the skin consisting of dense collagen fibers, that was originally described in German Shepherds. We previously mapped RCND to canine chromosome 5 (CFA5) with a highly significant Lod score of 16.7 (theta = 0.016). We have now narrowed the RCND interval, following selection and RH mapping of canine genes from the 1x canine genome sequence, to a small portion of CFA5 that corresponds to human chromosome 17p11.2. These sequences also allowed for the isolation of gene-associated BACs and the characterization of new microsatellite markers from each BAC. Ordering of markers and genes with regard to recombinants localizes RCND to an interval that corresponds to the interval on human 17p11.2 believed to contain the Birt-Hogg-Dube (BHD) locus. BHD is an inherited autosomal dominant human renal cancer syndrome that bears striking resemblance to RCND. Specifically, affected individuals experience similar skin tumors, uterine leiomyomas and renal neoplasms. The BHD gene has not yet been identified and we do not know if RCND and BHD are caused by mutations in the same or closely positioned genes. Using comparative genomics, we evaluated potential candidate genes in the region and have begun mutation screening in those considered most likely. This work underscores the emerging power of canine genomics to aid in the pursuit of genes causing mammalian disease.

Program Nr: 247 from 2002 ASHG Annual Meeting

**Biallelic germline MYH mutations in patients with multiple colorectal adenomas and cancer.** *S. Jones[1], J. Maynard[1], N. Al-Tassan[1], G.T. Williams[2], D.R. Davies[3], J.R. Sampson[1], J.P. Cheadle[1]*. 1) Institute of Medical Genetics, University of Wales College of Medicine, Cardiff, UK; 2) Department of Pathology, University of Wales College of Medicine, Cardiff, UK; 3) Department of Gastroenterology, University Hospital of Wales, Cardiff, UK.

We have recently shown that inherited defects of the base excision repair gene MYH predispose to multiple colorectal adenomas and carcinoma. We found that three affected siblings from family N were compound heterozygotes for the functionally compromised MYH missense variants, Y165C and G382D (Al-Tassan et al. Nat. Genet. 2002). We have now identified seven further families with multiple colorectal adenomas and biallelic germline MYH mutations: four are homozygous for truncating mutations, two are homozygous for Y165C and one is a Y165C/G382D compound heterozygote. As predicted from studies of the bacterial and yeast homolog of MYH, colorectal tumours from affected individuals display a significant excess of somatic G:C to T:A mutations, as compared to sporadic tumours. This novel autosomal recessive cancer predisposition syndrome is the first reported inherited disorder of base excision repair in humans.

Program Nr: 248 from 2002 ASHG Annual Meeting

**Mutations in fumarate hydratase cause uterine fibroids, cutaneous leiomyomas and aggressive renal neoplasia.**

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Uterine leiomyomas are the most common gynecological tumors in women of reproductive age, with a prevalence ranging from 20% to as high as 77%. Uterine leiomyomas constitute a major health issue for women associated with high morbidity due to abdominal pain, menorrhagia and infertility. The inherited predisposition to develop cutaneous and uterine leiomyomas, and renal cancer have been designated as hereditary leiomyomatosis and renal cell carcinoma (HLRCC). For the last four years we had the opportunity to evaluate the largest cohort of American families with HLRCC. 61% in our cohort of affected individuals had cutaneous leiomyomas and 82 % of affected women had uterine fibroids. We also found that 67% of women the affected women had hysterectomies of whom 52% had a hysterectomy by the age of 30. We have identified 16 individuals from 4 families with renal tumors;11 of whom died within 5 years of diagnosis. Renal tumors were unilateral, solitary, and metastatic at time of diagnosis. Most renal tumors had a papillary type II histology. Sequencing analysis revealed germline mutations in fumarate hydratase(FH) in 11/13 families. LOH studies in tumors suggest that FH functions as a tumor supressor gene. Mutations in FH are associated with leiomyomatosis and renal cancer in American families. Funded in part by DHHS#NO1-CO-12400.

Program Nr: 249 from 2002 ASHG Annual Meeting

**Diagnostic testing for mutations in the PTEN gene using direct sequencing.** *J.W. Heinz[1], R.T. Pilarski[2], C. Eng[2], T.W. Prior[1]*. 1) Pathology, The Ohio State University, Columbus, OH; 2) Clinical Cancer Genetics, The Ohio State University, Columbus, OH.

Mutations in PTEN, a tumor suppressor gene on 10q23, have been identified in Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRR) and Proteus syndrome (PS). Our laboratory provides a molecular diagnostic service for patients with CS, BRR, CS/BRR-like, Proteus and PS-like phenotypes. Since mutations occur throughout the gene, our testing strategy involves direct sequencing of the nine PTEN exons. From 0/0/2000 through 5/2002, we have tested 73 patients for the presence of mutations in the nine PTEN coding exons and consensus splice sites. Twelve assays (16%) were confirmations of research results of known mutations. Twenty-two (30%) samples were tested for the detection of known mutations in at-risk family members, representing 11 families. Five (23%) family members tested mutation positive. We have had 39 requests for total gene characterization and have identified 6 PTEN mutations. A variety of different mutations (missense, nonsense, splice site and frameshifting deletions and insertions), along with common polymorphic variants have been found in the PTEN gene. A spectrum of clinical manifestations were observed in our patients who tested positive for a PTEN mutation. Macrocephaly, benign breast, thyroid growths and skin/mucosal lesions were the most common findings. We conclude that direct sequencing is a sensitive method and can be adapted by a diagnostic laboratory for the detection of PTEN mutations, and that clinical features and family history can be used to identify those individuals most likely to carry a PTEN mutation.

Program Nr: 250 from 2002 ASHG Annual Meeting

**Germline missense and nonsense PTEN mutations result in splice aberrations in patients with Cowden and Bannayan-Riley-Ruvalcaba syndromes.** *M.J. Fernandez, X.P. Zhou, H. Hampel, G. Boru, C. Eng.* Human Cancer Genetics Program,Ohio State Univ, Columbus, OH.

Germline mutations in PTEN, encoding a dual specificity phosphatase tumor suppressor on 10q23, cause subsets of Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS) and Proteus syndrome. These 3 syndromes are clinically unrelated but may be allelic at the genetic level. There are >110 germline mutations reported for CS and BRRS. The germline mutational spectrum includes nonsense, missense, insertion, deletion, and splice site mutations. Nonsense and frameshift mutations are presumed to result in truncated protein, and hence loss-of-function. Splice site mutations presumably result in adjacent exon skipping and truncated protein, but most such mutations in PTEN have not been tested at the RNA or protein level. PTEN missense mutations have been shown to abrogate or reduce its lipid phosphatase activity resulting in loss of apoptosis or G1 arrest. We hypothesized that another mechanism of inactivation resulting from point missense and nonsense mutations is the mediation of aberrant splicing. We performed RT-PCR from RNA templates from 17 CS or BRRS patients with germline mutations. Of these 17, 12 demonstrated aberrant splicing. Of note, all resulted in exon 6 skipping and the 3'of exon 5 was fused with the 5' of exon 7. As expected, 2 splice site mutations resulted in aberrant splicing. Interestingly, IVS3-39A>G resulted in aberrant splicing as well. Further, aberrantly spliced transcripts were noted in those with germline missense (R130Q, L345V), nonsense (R130X, Q149X, R335X), microinsertion (c.246InsAAAG, c.955InsA) and microdeletion (c.955-8delACTT, c.957-9delT) mutations. No aberrant splicing occurred in control samples. Our data suggest that non-splice site mutations, even exonic point mutations and variants deep within an intron can result in aberrant splicing of PTEN transcript. Thus, more than one mechanism of inactivation may result from any single mutation. Quantitative RNA and protein analyses are in progress and may reveal further mechanisms of inactivation resulting from an exonic mutation.

Program Nr: 251 from 2002 ASHG Annual Meeting

**Downregulation of PPARg is a frequent and early event in follicular thyroid neoplasia.** *M.A. Aldred[1], O. Gimm[2], C. Hoang-Vu[2], H. Dralle[2], S. Jhiang[1], C. Eng[1]*. 1) The Ohio State University, Columbus, OH; 2) Martin-Luther-University, Halle-Wittenberg, Germany.

Follicular thyroid cancer (FTC) is relatively uncommon in the general population, but is an important component tumor of Cowden syndrome. Little is known about the etiology. LOH studies have identified allelic losses particularly on 3p, 7q, 10q and 17p. The most significant finding to date has been a translocation between PAX8 and PPARg at 2q31 and 3p25 respectively, detected in 5 of 8 FTCs in one study. This translocation places PPARg under the control of the PAX8 promoter, leading to upregulation at the mRNA level. However, the fusion protein apparently acts as a dominant negative inhibitor of normal PPARg function.

In a cohort of 19 mostly German FTCs, we detected a PAX8-PPARg fusion transcript in only 2 tumors. However, microarray expression studies revealed downregulation of PPARg in many of the remaining tumors. Semi-quantitative fluorescent RT-PCR analysis confirmed significant downregulation in 14/17 non-translocation samples. Furthermore, extension to a panel of goiters, follicular adenomas and hyperplastic thyroid tissue showed reduced expression of PPARg in 6/14 such benign samples. LOH of nearby microsatellite markers was detected in around one-third of FTCs but not in benign tissues. Mutation analysis of PPARg is ongoing, but no mutations have been identified so far. Three important conclusions can be drawn from our results. Firstly, the PAX8-PPARg translocation is less common amongst FTC tumors than originally thought, at least in some geographical areas, which limits its value as a diagnostic tool to differentiate FTCs from other benign and malignant thyroid conditions. Nevertheless, functional downregulation of PPARg is confirmed to be a key event in follicular thyroid carcinogenesis and may variously result from the dominant-negative fusion protein, from LOH or via other, as yet undetermined genetic or epigenetic events. Finally, the detection of reduced PPARg mRNA levels in a significant minority of benign lesions suggests that this is a very early event in follicular thyroid neoplasia and is a possible target for therapeutic intervention.

Program Nr: 252 from 2002 ASHG Annual Meeting

**Use of a human expression system and microarray facilitates analysis of genome-wide alterations in MMR and APC/b-catenin -modulated colorectal cancer models.** *Z. Yuan[1], T. Sotsky Kent[1], J. Trojan[2], T.K. Weber[1].* 1) Albert Einstein Col Medicine, NY, NY; 2) Johann Wolfgang Goethe University, Frankfurt, Germany.

**Introduction**: Pathways of malignant transformation in MMR- and APC/b-catenin-deficient colorectal cancer (CRC) await clarification. We employed cDNA microarray of a human expression system to compare gene expression profiles of CRC models of these two systems. **Methods**: MMR- and APC/b-catenin-deficient lines were engineered from a 293T human expression system with the following pathologic mutations: hMLH1-T117M, hMLH1-K618T, hMLH3-del Exon 7, APC-D1309, b-catenin-D45. MMR and APC protein expression were assayed by Western blot. MMR phenotype was assessed by functional assays and DNA sequencing. cDNA microarray was performed to compare hMLH1-MT to -WT, and APC and/or b-catenin-MT to -WT. Results were considered significant when expression ratios >1.5 were consistent in 4 of 5 experiments. Real-time PCR confirmed microarray results. **Results**: cDNA microarray identified 417 and 729 genes differentially expressed in MMR and APC/b-catenin-deficient systems, respectively. Significant expression ratios were detected in MMR-deficient lines in DNA repair, TGF-b/SMADs, apoptosis, and RAS/MAPK pathways. Oncogenes and tumor suppressor genes consistently differentially expressed in MMR-deficient lines included DLC, DOC-1, SWI/SNF2, and SWI/SNF3. In APC/b-catenin-deficient models, differential expression occurred in Wnt, cadherin, and apoptosis pathways. **Discussion**: We used a human expression system to demonstrate that, in MMR deficiency, TGF-b/SMADs signal transduction is blocked, leading to Ras/MAPK activation, and apoptosis inhibition. In the APC/b-catenin-modulated system, b-catenin levels increase due to decreased degradation, and Wnt and Cadherin signal transduction are increased. These changes promote cell proliferation, decreased mobility, and increased oncogene transcription. These data indicate cDNA microarray analysis of a controlled human expression system facilitates comparative analyses of transformation pathways in MMR- and APC-deficient CRC models. This technique provides a unique opportunity to study genome-wide alterations associated with malignant transformation in CRC.

Program Nr: 253 from 2002 ASHG Annual Meeting

**Haplotypes predict therapeutic ovarian cancer response.** *R. Mirhashemi[1], J.F. Arena[1], N. Lambrou[1], J. Arboleda[1], M. Hunt[1], H. Averette[1], M. Penalver[1], M. Thomas[2], Z. Gaskin[2], S. Gunjupulli[2], V. Kondragunta[2], P.K. Nachimuthu[2], P. Visu[2], S. Natarajan[2], S. Gunturi[2], T. Frudakis[2]*. 1) Dept OB/GYN, D-52, Univ Miami Sch Medicine, Miami, FL; 2) DNAPrint Genomics, Inc., 900 Cocoanut Ave. Sarasota, FL 34236.

Thirty five percent of Ovarian Cancer (OC) patients fail to respond to first-line combination paclitaxel (taxol) and carboplatin (TC) therapy. Poor first line chemotherapy response portends significantly higher patient morbidity and mortality. Because OC patients exhibit wide variability in the TC metabolism, it is possible that some or all of this variable response can be explained in pharmacogenetic terms. To determine whether common polymorphisms are associated with variable TC response, we applied novel analytics and data resources for a candidate gene survey of several drug metabolism genes. After genotyping 42 ovarian cancer patients at 112 SNPs in xenobiotic metabolism genes, we used an empirical (lexigraphical) approach to screen all possible SNP combinations to identify those with haplotype alleles that were genetic features of variable TC response. At the present time, we have identified two such combinations. The first is comprised of 3-SNPs spanning 9.6Kb of the 3 half of the CYP3A4 gene (avg. $p<0.004$). The second combination contained 3-SNPs spanning a 7.5Kb segment of the 3 half of the CYP2C8 gene (avg. $p<0.008$). Approximately 61% (25/41) of the patients exhibited a positive Overall TC response during first line treatment. These TC response rates are roughly comparable to the 65% average positive response rate described in previous reports for TC combination therapy. Using an overall clinical response criteria for evaluation of response over the treatment line the accuracy of the responder classification was 96%, the sensitivity of the non-responder classification was 90% and the test overall efficiency was 81%. Our results suggest that first-line TC response is largely a function of xenobiotic metabolism in OC patients, rather than tumor type or stage, and that CYP3A4 and CYP2C8 haplotype combinations are potentially useful for pre-screening OC patients for an individualization of chemotherapy.

Program Nr: 254 from 2002 ASHG Annual Meeting

**Cox-2 gene promoter haplotypes and prostate cancer susceptibility.** *R.K. Panguluri[1], S. Wang[1], W. Chen[1], W. Issacs[2], C. Ahaghotu[3], R.A. Kittles[1,3].* 1) National Human Genome Center at Howard University, Washington, DC; 2) Johns Hopkins University Oncology Center, Baltimore, MD; 3) Department of Urology, Howard University Hospital Washington, DC.

Archidonic acid metabolizing enzymes such as Cyclooxygenase-2 (Cox-2) are implicated in prostate cancer development and/or progression. The expression of Cox-2 is strongly correlated with increased tumor microvasculature. Cox-2 also plays an important role in inhibiting apoptosis, stimulating angiogenesis and promoting tumor cell metastasis and invasion. The Cox-2 promoter consists of various transcriptional regulatory elements. Polymorphisms in the 5' regulatory regions of the Cox-2 gene may have profound effects on the expression of the enzyme. To this end, approximately 1,400 nucleotides of the Cox-2 promoter region were screened using dHPLC. Four novel SNPs, -1285A/G, -1265G/A, -899G/C and -297C/G, were detected and confirmed by direct sequencing. Three clinical populations consisting of African American, Nigerian, and European American prostate cancer cases and age and ethnicity matched controls were genotyped for three of the promoter SNPs. Haplotypes were generated using the EM algorithm. Association analyses of prostate cancer and related clinical features were performed by contingency and regression analyses using single markers and haplotypes. Association analyses of single markers revealed that the -1285 and -899 SNPs were associated with prostate cancer in European Americans and Nigerians ($p<0.04$) and also aggressiveness of cancer in European Americans ($p<0.01$). Six haplotypes were observed in African American and European American cases, while only four were found in control populations. Interestingly, all three SNPs were in strong linkage disequilibrium ($D'>0.90$; $p<0.001$) in Nigerians and African American cases but not in European American cases. One of six haplotypes was strongly associated with early onset prostate cancer (<65 years of age) (OR= 6.7; $p=0.0001$) in African Americans and Nigerians. These data suggests that SNPs in the Cox-2 promoter may influence the risk and development of prostate cancer and haplotypes may be informative for screening at-risk populations.

Program Nr: 255 from 2002 ASHG Annual Meeting

**Mutations in CHK2 associated with prostate cancer risk.** *X. Dong[1], L. Wang[1], K. Taniguchi[1], X. Wang[1], J.M. Cunningham[1], S.K. McDonnell[2], C. Qian[1], A.F. Marks[1], S.L. Slager[2], B.J. Peterson[2], D.I. Smith[1], J.C. Cheville[1], M.L. Blute[3], S.J. Jacobsen[2], D.J. Schaid[2], D.J. Tindall[3], S.N. Thibodear[1], W. Liu[1]*. 1) Division of Experimental Pathology, Dept of Laboratory Medicine and Pathology; 2) Dept of Health Sciences Research; 3) Dept of Urology, Mayo Clinic/Mayo Medical School.

The DNA damage-signaling pathway has been implicated in the development of nearly all human cancers. However, the genetic defects and the mechanisms of this pathway in prostate carcinogenesis are poorly understood. Here we show that CHK2, the upstream regulator of p53 and the mutation target in Li-Fraumeni syndrome (LFS), is mutated in a subset of prostate cancer. Among 3 groups of sporadic prostate cancers, 13 CHK2 germ-line mutations (9 of which were unique) were identified in 262 patients. Screening for mutations in 2 affected individuals from each of 150 familial prostate cancer families revealed 9 CHK2 mutations (5 unique) in 9 families. These mutations included two frameshift and three missense mutations and segregated with prostate cancer within these pedigrees. Importantly, 9 of the 11 unique CHK2 mutations identified in the sporadic and familial cases were not detected among 423 unaffected men, suggesting a pathological effect of CHK2 mutations in prostate cancer development. Functional analyses of the two frameshift mutations in EBV-transformed cell lines, using RT-PCR and Western blot analysis, showed abnormal splicing for one mutation and dramatic reduction of CHK2 protein levels in both cases. Overall, our data indicates that mutations in CHK2 may contribute to prostate cancer risk and that the DNA damage-signaling pathway may play an important role in the development of prostate cancer.

Program Nr: 256 from 2002 ASHG Annual Meeting

**Complete dissection of a human quantitative trait locus: Factor VII plasma levels and the *F7* structural gene.** *J. Blangero[1], L. Almasy[1], J.C. Souto[2], J. Fontcuberta[2], J.M. Soria[2].* 1) Dept Genetics, SW Foundation Biomedical Res, San Antonio, TX; 2) Hospital Sant Pau, Barcelona, Spain.

The localization of quantitative trait loci (QTLs) influencing risk factors for common disease is now routine. However, the identification of QTLs and the elucidation of their allelic architecture presents a formidable challenge. We recently developed a Bayesian quantitative trait nucleotide (BQTN) approach designed to statistically identify the functional polymorphisms within positional candidate genes as a prelude to more expensive molecular functional characterization. Using this method, we have for the first time successfully dissected a human QTL, including the complete description of its allelic architecture.

In a genome scan of 398 individuals in 21 families, we localized a QTL influencing Factor VII (FVII) plasma levels (a risk factor for thrombosis) to a location on chromosome 13 coincident with the FVII structural gene. Comprehensive resequencing of this 15 kb gene in 40 individuals identified a total of 47 polymorphisms. Using the BQTN method and its implementation on a parallel computing cluster, we examined all reasonable models of gene action. This method intrinsically accounts for the many models considered by using a Bayesian model averaging approach and can accurately estimate posterior probabilities of functionality of polymorphic sites. Our results identify at least five functional variants in this gene influencing variation in FVII plasma levels. The variants include both regulatory elements in evolutionarily conserved regions and amino acid changes. Conditioning upon this multivariant model eliminates the evidence for linkage, suggesting that these variants completely account for the QTL's influence on FVII levels. Our result represents a successful elucidation of a human QTL starting from localization through a genome-wide linkage screen to investigation of a positional candidate gene to complete enumeration of multiple functional variants. The pattern of disequilibrium among variants in this gene also suggests that linkage disequilibrium-based approaches to QTL dissection would not have been sufficient to identify all functional sites.

Program Nr: 257 from 2002 ASHG Annual Meeting

**Evidence for a gene influencing serum bilirubin on chromosome 2q telomere: a genome-wide scan in the Framingham Offspring Study.** *J.-P. Lin[1], L.A. Cupples[2], P.W.F. Wilson[3], C.E. Jaquish[1], C.J. O'Donnell[4]*. 1) DECA/NHLBI, NIH, Bethesda, MD; 2) Boston University School of Public Health, Boston, MA; 3) Boston University School of Medicine, Boston, MA; 4) NHLBI/Framingham Heart Study, Framingham, MA.

There is an inverse relationship between serum bilirubin concentrations and risk of coronary artery disease (CAD). The strength of the association between serum bilirubin and CAD was similar to that of smoking, systolic blood pressure, and HDL-cholesterol. We carried out a 10 cM genome-wide scan in a community-based Caucasian cohort, the Framingham Heart Study. Our study sample consisted of 330 families with 1308 individuals being both genotyped and phenotyped, including 913 sibling pairs, 713 cousin pairs and 92 avuncular pairs. Using variance-component methods implemented in SOLAR, our genome-wide linkage analysis by an empirical P-value method demonstrated significant evidence of linkage of serum bilirubin to chromosome 2q telomere with a LOD score of 3.8 (p=0.00001) at location 243 cM. The peak multipoint LOD score is located at about 1 cM away from the location of the Uridine diphosphate glycosyltransferase 1 (UGT1A1) gene. UGT1A1, which catalyzes the conjugation of bilirubin with glucuronic acid and thus enhances bilirubin elimination, has been considered as an important candidate gene of serum bilirubin. Gilbert syndrome, which has a population frequency of 2-19%, is mainly due to a TA insertion at the promoter region of UGT1A1 gene and results in a reduced level of expression of the gene, and thus increased serum bilirubin concentrations. This gene was mapped to chromosome 2q37. Only one other region in the genome produced a multipoint LOD score greater than 1 (LODs = 1.3). Our study supports the contention that UGT1A1 may be the major gene controlling serum bilirubin levels in the population.

Program Nr: 258 from 2002 ASHG Annual Meeting

**Linkage and association of BMI to markers on 7q in the Old-Order Amish.** *G.J. Papanicolaou[1], P. Platte[2], J. Johnston[3], K. Doheny[4], E.W. Pugh[4], M.-H. Roy-Gagnon[1,5], A.J. Stunkard[6], C. Francomano[7], A.F. Wilson[1].* 1) Genometrics Sect., NHGRI, NIH, Balt., MD; 2) Biological & Clinical Psych., U. Wuerzburg, Germany; 3) NHGRI, NIH, Bethesda, MD; 4) CIDR, JHU SOM, Balt., MD; 5) Dept. Epidemiology, JHSPH, Balt., MD; 6) Dept. Psychiatry, U. PA, Phila., PA; 7) NIA, NIH, Balt., MD.

The epidemic increase in obesity, whether due to increased food intake or decreased calorie expenditure, is thought to have a genetic component, with most estimates of heredity ranging between .25 and .40. As part of an ongoing study of traits related to obesity in the Old-Order Amish, seven two- and three- generation families totaling 157 individuals were assessed for 21 obesity related traits, including body mass index (BMI) and %BMI (BMI adjusted for age and sex), and genotyped at CIDR with a modified CHLC 9 markers set consisting of 384 short tandem repeat markers with an average distance of 9 cM. In this sample, the estimates of heritability ranged from 0.16 to 0.31 for BMI and from 0.40 to 0.52 for %BMI. Model independent linkage analysis identified candidate regions on chromosomes 1, 5, 7, 8 and 11. Several markers on 7q were significant for both BMI and %BMI ($p < 0.001$), with and without the inclusion of covariates. This region (7q) contained the structural locus for leptin and was considered to be the primary candidate region. Additional flanking markers corroborated the original findings and linkage results were, on balance, more significant than those of the original markers. Tests of intrafamilial association for alleles at markers in this candidate region were significant at similar levels. Although there is some evidence for linkage and association in the region containing leptin, there appears to be stronger evidence for linkage to BMI within a 10-15 cM region downstream, flanked by markers D7S1804 and D7S3070, suggesting that this region may be responsible, at least in part, for variation of BMI and %BMI in the Old-Order Amish. Additional results include significant values ($p < 0.001$) for traits affecting measures of hip and waist, diastolic b.p., and fat mass, and levels of glucose, insulin, and free t3 and t4 among others.

Program Nr: 259 from 2002 ASHG Annual Meeting

**International Endogene Study finds strong evidence of susceptibility loci for endometriosis at two genomic regions.** *J. Wicks[1,2], D. Zabaneh[3], S.A. Treloar[1,2], R. Hadfield[4], G. Dawson[3], A. Lambert[3], B. Haddon[1,2], I. Mackay[3], D.E. Weeks[5], D.T. O'Connor[2], D. Schuette[3], N. Gough[1], M. Smith[6], A. Douglas[1], G. Montgomery[1,2], S. Bennett[3], D.H. Barlow[4], N.G Martin[1,2], S.H. Kennedy[4].* 1) Cooperative Research Centre for Discovery of Genes for Common Human Diseases; 2) Queensland Institute of Medical Research; 3) Oxagen Ltd; 4) University of Oxford; 5) University of Pittsburgh; 6) Cerylid Biosciences Ltd.

Endometriosis is a complex disease affecting up to 10% of women in their reproductive years. The disease is defined as the presence of tissue resembling endometrium in extra-uterine sites, most commonly the ovaries and peritoneum. The only reliable diagnostic test is inspection of the pelvis at laparoscopy. The main clinical manifestations are painful periods, pain during intercourse, pelvic pain and infertility. Evidence that genes influence susceptibility is now extensive, and includes a large-scale twin study (Treloar et al, 1999).

The International Endogene Study was recently formed by a collaboration between an Australian and a UK based group to create a large combined resource for the mapping of genes for endometriosis (Treloar et al, 2002). The combined resources currently comprise over 1100 affected sibships (mostly ASPs) for genome scanning to identifying linked regions, and an additional 1200 triads and 560 independent cases for fine mapping and SNP association analysis. There are plans to recruit additional cases and controls to a total of 2000. This represents one of the largest resources ever assembled for finding genes for a complex disorder. The total number of ASP families provides approximately 80% power to detect susceptibility loci with locus-specific $l_s$ down to 1.33 (Risch, 1990).

To date a total of 881 ASP pedigrees have been genotyped using a 400 microsatellite marker set with ~10cM coverage. Data are being analyzed using Genehunter, and two regions of suggestive linkage have so far been identified with MLS scores of 3.76 and 3.66. Conservative thresholds for suggestive and significant linkage for the MLS are 2.45 and 3.93 respectively (Lander & Kruglyak, 1995; Nyholt, 2000).

Program Nr: 260 from 2002 ASHG Annual Meeting

**Identification of a Novel Late Onset Alzheimer's Disease Gene on Chromosome 10.** *S.G. Younkin[1], J. Ronald[1], S. Jain[1], M. Hella[1], A. Singleton[1], H. Asahara[1], E. Gnida[1], M. Baker[1], J. Adamson[1], D. Fadale[1], L. Younkin[1], N. Graff-Radford[2], M. Hutton[1], N. Ertekin-Taner[1,3].* 1) Department of Neuroscience, Mayo Clinic, Jacksonville, FL; 2) Department of Neurology, Mayo Clinic, Jacksonville, FL; 3) Department of Neuroscience, Mayo Clinic, Rochester, MN.

At least three studies have shown through linkage analysis that a locus on chromosome 10 (Ch10) contributes to risk for late-onset Alzheimer's disease (LOAD). In our study using plasma Ab42 levels as a quantitative surrogate phenotype in extended LOAD pedigrees, we obtained linkage to a locus on Ch10 at 81 cM. Linkage to the same locus was obtained in a genome screen of LOAD sib-pairs. Together these studies provide strong evidence for a major locus on Ch10 that increases the risk for LOAD by elevating Ab42. To identify the Ch10 gene responsible for the linkage signal at 81 cM, we evaluated single nucleotide polymorphisms (SNPs) for association with Ab42 in LOAD families. Using the same 10 LOAD families that were used to obtain linkage to Ab42, we identified a novel Ch10 gene with 2 SNPs (in tight linkage disequilibrium) that showed highly significant association ($p<0.00002$) with plasma Ab42. This association is robust because we were able to replicate it in a separate set of 12 additional LOAD families, and when all 22 families were combined significance improved to $p=0.000002$. The association clearly accounts for the linkage signal at 81 cM because we found no evidence for additional linkage ($p >0.3$) when we performed linkage analyses conditional upon the association. To test the two SNPs for association with LOAD, we examined a publicly available collection of LOAD sib-pairs and detected significant association ($p < 0.02$). To fine map and bound the region of association, 3-10 kb haplotype blocks spaced at 250 kb intervals were analyzed throughout the candidate gene. Maximal association to Ab42 was observed with the haplotype block containing the 2 SNPs with highly significant association. Association declined progressively on either side of this block, bounding the association to variants located within the gene. Our results identify a novel LOAD risk gene that accounts for the Ab42 linkage signal on chromosome 10.

Program Nr: 261 from 2002 ASHG Annual Meeting

**A Genomic Screen For Dementia in Amish Families.** *J.L. Haines[1], K.K. Nicodemus[2], A. Crunk[1], L. McFarland[1], P.C. Gaskell[2], S. West[2], J. Vance[2], G. Jackson[3], M.A. Pericak-Vance[2].* 1) Program Human Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) Henry Ford Hospital, Detroit, MI.

Alzheimer disease (AD) is the leading cause of dementia in the elderly. Four genes underlying AD have already been identified, three of which (APP, PSEN1, PSEN2) represent rare, mostly early-onset forms of the disease. The fourth gene, APOE, modulates risk and onset in both early and late onset AD. Collectively, these genes represent less than 50% of the overall genetic risk of AD. Consensus from several genomic screens of outbred populations point to genomic regions on chromosomes 9, 10, and 12 as likely locations for some of the remaining genes. A recent study of a small inbred Arab-Israeli population also pointed toward chromosomes 9 and 12. We have previously studied the Amish populations in the Midwest and demonstrated a familial effect on late-onset dementia that is not accounted for by APOE. We performed a genomic screen in eight highly complex pedigrees with 123 total sampled individuals (42 affected), using a standard set microsatellite markers at ~10 cM density. Given the complex nature of the pedigrees, five screening statistics from SIMWALK 2.0 were used. Ten markers on chromosomes 1, 7, 9, 10, and 14, defined 7 different regions that met our initial screening criteria of a P value < 0.10 on more than one statistic. The strongest results were on chromosome 10p (15-40 cM on the Marshfield map) where three adjacent markers provided P values <0.002. This region is different than the previously proposed regions on chromosome 10, all of which are on 10q. These data suggest that at least one novel locus for dementia exists in the Amish population.

Program Nr: 262 from 2002 ASHG Annual Meeting

**Genetic Dissection of Linkage to Late-onset Alzheimer Disease.** *J.M. Olson, K.A.B. Goddard, D.M. Dudek, Y. Song.* Epid/Biostat,MetroHlth Med Ctr, Case Western Reserve Univ, Cleveland, OH.

Alzheimer disease (AD) is the most common form of dementia in the elderly and is believed to be genetically complex. Locus heterogeneity presents a challenge in the analysis and interpretation of linkage results. Recently, we developed a model-free linkage method that allows covariates to be included in an affected-sib-pair (ASP) linkage analysis. This method accounts for locus heterogeneity that is measured by covariates, thereby allowing the discovery of linkage effects that might otherwise be obscured. We recently reported two large linkage effects that also show strong epistasis, suggesting the existence of a subgroup of AD linked to both 20 and 21 and characterized by late age. We now propose a systematic approach for partitioning a set of ASPs into genetically homogeneous subsets using allele-sharing and other phenotypic information. After first identifying large covariate signals and evaluating epistasis, we compute a linkage probability for each ASP and assign the ASP to either the "linked" or "unlinked" subgroup. Serial application of the method to the "unlinked" subgroup is then used to separate additional subgroups linked to other genomic regions. We applied the method to ASPs collected through the NIMH AD Genetics Initiative and obtained a model of late-onset AD consisting of at least four subgroups: 1) Middle-onset, due primarily to the action of ApoE but with substantially earlier onset in the presence of loci on 18 and 22, 2) Late-onset-1, due to loci on 10 and 8p, and 3) Late-onset-2, genetically heterogeneous but due in part to a locus on 16, and 4) Very-late-onset, due to the joint action of loci at 20p and 21p. To control for multiple testing, we calculated false discovery rates; each of these effects (plus a few more) had an FDR < .25. The partitioning of a complex disease into genetically homogeneous subsets is expected to facilitate both fine mapping efforts and candidate gene selection. For example, we hypothesize that loci on 18 and 22 act biologically to enhance the detrimental effects of the ApoE4 allele. In summary, we expect that by partitioning complex disease data sets we can greatly accelerate the process of gene discovery.

Program Nr: 263 from 2002 ASHG Annual Meeting

**Linkage analysis of extremely discordant and concordant sibling pairs identifies QTL that influence variation in a human personality trait.** *J. Fullerton[1], M. Cubin[1], A. Bhomra[1], S. Davidson[1], S. Miller[1], M. Turri[1], C. Dolby[1], R. Mott[1], C. Wang[2], H. Tiwari[2], D. Allison[2], M. Neale[3], C. Fairburn[4], G. Goodwin[4], J. Flint[1]*. 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; 2) Department of Biostatistics, Ryals Public Health Building, University of Alabama at Birmingham, Alabama 35294; 3) Department of Psychiatry, Virginia Commonwealth University, Virginia Institute for Psychiatric & Behavioral Genetics, Richmond VA; 4) Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford, United Kingdom.

Several theoretical studies have suggested that large samples of randomly ascertained siblings can be efficiently used to ascertain phenotypically extreme individuals and thereby increase power to detect genetic linkage in complex traits. Here we report a genetic linkage scan using 87 extreme discordant and 190 extreme concordant sibling pairs, selected from 34,000 sibships in the south west of England who completed a personality questionnaire. We carried out a genome wide scan for quantitative trait loci (QTL) that influence variation in the personality trait of neuroticism (or emotional instability) and found evidence for linkage to chromosomes 1p, 7p, 11q, 12q and 13q. Using a composite test of deviation from expected identity-by-descent (IBD) and regression of IBD status on phenotype, the P-values for these loci are $1.14X10^{-4}$ (1p) $3.26X10^{-6}$ (7p), $3.83X10^{-5}$ (11q) and $3.4X10^{-5}$ (12q). One locus, on chromosome 1, is syntenic with that reported for QTL mapping of rodent emotionality, an animal model of neuroticisim. This is the first report of the successful application of extreme selection strategies to identify QTL and suggests that animal and human QTL may be homologous.

Program Nr: 264 from 2002 ASHG Annual Meeting

**Genome-wide scan of 153 bipolar multiplex pedigrees ascertained through the NIMH Genetics Initiative for Bipolar Disorder.** *M.G. McInnis[1], D.M. Dick[2], D.F. MacKinnon[1], F.J. McMahon[3], J.B. Potash[1], J.I. Nurnberger[2], E.S. Gerson[3], H.J. Edenberg[2], T. Reich[4], J.P. Rice[4], S.G. Simpson[1], V.L. Willour[1], J.R. DePaulo[1], P.P. Zandi[1], T. Foroud[4].*
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A 4 center genetic collaborative effort has studied bipolar (BP) multiplex pedigrees ascertained through a bipolar I proband with at least one BPI affected sibling and 2 additional living siblings. The evaluation used the Diagnostic Interview for Genetic Studies (DIGS), which generates both DSM and RDC diagnoses. The current analyses consist of 513 polymorphic markers in 899 subjects from 153 pedigrees. The sample included 334 BPI, 115 BPII (with recurrent major depression [RMD]), 57 schizo-affective manic (SAM), and 141 subjects with recurrent major depression. Analyses were performed under 3 affection status models (ASM): ASM I included BPI and SAM (391 subjects); ASM II included BPI, SAM, and BPII with RMD (506 subjects); ASM III included BPI, SAM, BPII with RMD, and RMD (647 subjects). The method of analysis was a nonparametric analysis of allele sharing among affected relative pairs; ASMI 284 pairs, ASMII 529 pairs, and ASMIII 894 affected relative pairs. There were 12 regions identified with nominally increased evidence of allele sharing among relative pairs. The 3 most significant regions were on chromosome 16p13 (p=0.0004), 20p12 (p=0.0037), and 6q24 (p=0.0056). The chromosome 16 and 20 findings have been reported previously in bipolar disorder and the chromosome 6 region has been reported in a genetic study of schizophrenia.

Program Nr: 265 from 2002 ASHG Annual Meeting

**Genome-wide significant linkage to chromosome 9 for panic disorder and anxiety in Iceland.** *T.E. Thorgeirsson[1], H. Oskarsson[2], N. Desnica[1], J. Pop Kostic[1], J.G. Stefansson[3], H. Kolbeinsson[3], E. Lindal[3], N. Gagunashvili[1], M.F. Frigge[1], A. Kong[1], K. Stefansson[1], J. Gulcher[1]*. 1) CNS Division, Decode Genetics, Reykjavik,Iceland; 2) Therapeia, Reykjavik, Iceland; 3) Landspitalinn-University Hospital, Reykjavik, Iceland.

**Introduction:** The results of the first genome-wide screen for anxiety disorders in the Icelandic population are described. The study aims to locate anxiety susceptibility genes utilizing the extensive genealogical records and the relative homogeneity of the Icelandic population. **Methods:** Participants were recruited in two stages: 1) Initial case-definition by a population screening for anxiety disorders, using the Stamm-Screening Questionnaire (SSQ), and by aggregation into extended families with the help of deCODE's Genealogy Database; 2) Those fulfilling diagnostic and family aggregation criteria received a further diagnostic work-up based on the Composite International Diagnostic Interview (CIDI). Screening for anxiety in close relatives also identified additional probands within the families. For linkage analysis affected families were defined by an Index Case with Panic Disorder and relatives with any anxiety disorder. Genotypes for 1000 microsatellite markers were used in an affecteds-only analysis and non-parametric lod scores calculated using the program Allegro. The average intermarker distance was ~4 cM, but ~1 cM in the linked region as additional markers from deCODE's high-resolution genetic map were included to increase the information content. **Results:** Linkage analysis of 26 extended families, each with at least one individual suffering from Panic Disorder, resulted in a genome-wide significant lod score for a region on chromosome 9. **Discussion:** Anxiety disorders encompass a constellation of conditions, including panic disorder, phobic disorders, and general anxiety disorder, all exhibiting complex interaction between environmental and genetic factors. The linkage results may be relevant to anxiety as a whole, because family members with no Panic Disorder but other forms of clinical anxiety are included in the linkage analysis.

Program Nr: 266 from 2002 ASHG Annual Meeting

**The DNA-based shape, length and banding pattern of interphase chromosomes.** *U. Claussen[1], J. Lemke[1], J. Claussen[1,2], I. Chudoba[3], P. Muehlig[4], K. Sperling[2].* 1) Institute of Human Genetics and Anthropology, Friedrich-Schiller-University, Kollegiengasse 10, D-07740 Jena, Germany; 2) Institute of Human Genetics, Humboldt-University, Augustenburger Platz 1,; 3) MetaSystems GmbH, Robert-Bosch-Str. 6, D-68804 Altlussheim, Germany; 4) Institute of Molecular Biotechnology, Beutenbergstr. 11, D-07745 Jena, Germany.

Chromosomes in interphase nuclei analysed with currently available techniques do not present any recognizable structures such as bands, centromeres, telomeres, or specific shapes. Microirradiation experiments and molecular cytogenetic investigations with whole chromosome paints and region specific microdissection probes have confirmed a territorial organization of chromosomes in interphase nuclei. Until now, however, their structure is not well understood. Using laser scanning microscopic examination and the high-resolution DNA-based multicolour banding (MCB) technique, we have generated a banding pattern and have determined the length of human chromosome 5 in lymphocyte interphase nuclei, and in nuclei of HeLa cells arrested at different phases of the cell cycle. The shape and MCB pattern of chromosome 5 in interphase nuclei is similar to that of metaphase chromosome 5 at all stages of the cell cycle. The length of the chromosome axis is comparable to that of a metaphase chromosome at a 600-band resolution. Therefore, the concept of chromosome condensation during mitosis has to be reassessed. Based on the MCB technique, interphase chromosomes can be used to identify chromosome aberrations which is of fundamental interest in cytogenetics.

Program Nr: 267 from 2002 ASHG Annual Meeting

**Nuclear matrix attachment regions in an olfactory receptor gene cluster.** *M.A. Goldman, A.A. Skrenchuck.* Department of Biology, San Francisco State University, San Francisco, CA.

Nuclear matrix attachment regions (MARs), also called scaffold attachment regions (SARs), may reflect the boundaries of chromatin domains or chromatin loops. Such domains could provide an environment for the coordinate developmental regulation of clusters or sets of functionally-related genes. Olfactory receptor (OR) genes most likely represent the largest superfamily in the vertebrate genome. All human chromosomes, excluding 20 and Y, have olfactory receptor gene clusters, most of which are in clusters of 6 to 138 genes. We have investigated the organization of OR loci with respect to MARs in order to test the functional clustering model and to further understand the organization of this interesting gene family. Here, we report our studies of the olfactory receptor cluster and the adjacent domain containing other genes expressed in brain, in chromosome region 11p15.4. Our localization of MARs using computational methods (MAR-Finder) support this model. Moreover, we performed matrix binding assays on two of the MARs identified computationally and both were confirmed. Acknowledgment: This research was supported in part through a grant from the National Institutes of Health, MBRS SCORE Program, Grant #S06 GM52588, and a Research Infrastructure in Minority Institutions award from the National Institutes of Health, Grant #5 P20 RR11805.

Program Nr: 268 from 2002 ASHG Annual Meeting

**LINE repeats are associated with the spread of X inactivation.** *A. Sharp[1], W. Tapper[2], P. Strike[3], D. Robinson[1], P. Jacobs[1]*. 1) Wessex Regional Genetics Lab, Salisbury, UK; 2) Human Genetics Division, Southampton University, UK; 3) Salisbury District Hospital, UK.

Recently Mary Lyon proposed that Long Interspersed Nuclear Elements (LINEs) might facilitate the spread of X inactivation in cis, a hypothesis supported by the distribution of LINEs on the X chromosome. As the distribution of LINEs is a constant property of each chromosome, Lyon's repeat hypothesis predicts that the spread of X inactivation into an autosome should be an intrinsic property dependent upon LINE content. We studied 5 unbalanced X;autosome translocations to test this hypothesis. We determined the transcriptional status of 33 genes on 4 translocated autosomes by RT-PCR and/or methylation analysis. 19 genes were classified as inactive whilst 14 were partially or fully active. Gene sequences and regions extending 10kb upstream were extracted from The Human Genome Browser and analysed using RepeatMasker and Tandem Repeats Finder. We observed a significant enrichment of LINEs around autosomal genes silenced by the spread of X inactivation. The difference in LINE content between active and inactive genes was greatest when 2kb of 5' sequence was included ($P<0.01$), but diminished with additional upstream sequence, suggesting that sequences within and immediately upstream of genes influence susceptibility to X inactivation. Long Terminal Repeats (LTRs) were also associated with inactive genes ($P<0.01$). Inactivation was not associated with SINEs, tandem repeats, low complexity DNA or GC content. Our results provide strong support for Lyons hypothesis that LINEs are booster elements which mediate the spread of X inactivation. The distribution of LINEs within the genome explains the variable spread of X inactivation which is seen in X;autosome translocations. The association of LTRs, which are mainly of viral origin, and LINEs with inactivated genes fulfils Lyons prediction that X inactivation might represent a form of repeat-induced silencing. Thus our data indicate that X inactivation may have originally evolved from a genome defence mechanism against the invasion of transposable elements, ascribing functional significance to what is often referred to as "junk DNA".

Program Nr: 269 from 2002 ASHG Annual Meeting

**Segregation of natural and artificial human chromosomes.** *M.K. Rudd, H.F. Willard.* Case Western Reserve Univ. and Univ. Hospitals of Cleveland. Cleveland, OH.

Human artificial chromosomes provide a tool to define the minimal sequence requirements for centromere function and to examine factors involved in proper chromosome segregation. In humans, alpha satellite has been implicated in centromere activity by genetic, genomic and functional studies and is competent to provide centromere function in artificial chromosomes. However, it is not known whether alpha satellite provides all of the sequences necessary for faithful chromosome segregation. We have studied 8 cell lines containing artificial chromosomes with de novo centromeres derived from either chromosome 17 (D17Z1) (n=5) or X chromosome (DXZ1) (n=3) alpha satellite. To study chromosome segregation directly, we used an anaphase assay that monitors both natural and artificial chromosome segregation in individual cell divisions using FISH. We scored ~44000 and ~60000 segregation events involving artificial or natural chromosomes, respectively. The mean missegregation rate for DXZ1 artificial chromosomes was 2.2% per cell division, while that of the natural X was 1.2% in the same cells. Similarly, the mean missegregation rate for D17Z1 artificial chromosomes was 3.0% per cell division, while that of natural chromosome 17s was 0.4%. Thus, overall, artificial chromosomes missegregated more often than natural chromosomes ($p<.0001$). Notably, the behavior of D17Z1 artificial chromosomes was heterogeneous. Three of the five artificial chromosomes tested missegregated at a rate of 1.6-2.0%, while two other lines showed rates of 9.7% and 4.4% per division. Interestingly, the type of segregation error also varied between artificial and natural chromosomes. Whereas nondisjunction accounted for 85% of natural chromosome missegregation, anaphase lag was responsible for the majority (56%) of artificial chromosome missegregation. These differences could reflect either structural or epigenetic differences among the artificial chromosomes tested and/or between natural and artificial chromosomes. We conclude that artificial chromosomes will be a useful model for studying factors causing segregation defects.

Program Nr: 270 from 2002 ASHG Annual Meeting

**Parent of origin and timing of de novo Robertsonian translocation formation.** *R. Bandyopadhyay[1], A. Heller[2], C. McCaskill[1], S.A. Berend[3], S.L. Page[4], L.G. Shaffer[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Institute for Human Genetics and Anthropology, Jena, Germany; 3) Genzyme Genetics, Santa Fe, NM; 4) Stowers Institute for Medical Research, Kansas City, MO.

Robertsonian translocations (ROBs) are the most common rearrangements in humans, occurring between acrocentrics 13, 14, 15, 21 and 22. ROBs are classified into 2 groups, common and rare, depending on their frequency. For this study, we have used 29 cases of de novo ROBs, including 19 common [15 rob(13q14q), 4 rob(14q21q)] and 10 rare [1 rob(14q22q), 2 rob(15q22q), 3 rob(15q21q), 4 rob(14q15q)]. We also report on the combined data set of 35 common ROBs from two other studies. All common ROBs showed breakpoints within the regions previously published. Somatic cell hybrids were used to identify the parental origin of each ROB. Of 15 rob(13q14q), 12 were maternal, 1 was paternal and 2 were postzygotic consisting of one paternal and one maternal chromosome. For rob(14q21q), both chromosomes were maternal in all cases. Of 4 rob(14q15q), 1 was maternal, 2 were paternal and 1 was posyzygotic. Rob(14q15q)s are diverse in their timing of formation, since both meiotic and mitotic formation was observed, and have variable breakpoint locations. The remaining rare ROBs were all maternal. Based on statistical analysis of the common ROBs, we can reject a mitotic model of formation in favor of a meiotic model ($c^2_2 = 35.5$, $P < 0.0001$) for this study and over all studies ($c^2_2 = 131.6$, $P < 0.0001$). Analyses of the rare ROBs showed that the majority were comprised of two maternal chromosomes and favored a meiotic model of formation ($c^2_2 = 11.4$, $P < 0.005$). Thus, both classes of ROBs occur predominantly during meiosis and the common ROBs occur primarily during oogenesis. The common ROBs likely form through a specific mechanism, distinct from the rare ROBs. We speculate that the common ROBs form through homologous recombination of shared sequences on chromosome 13, 14, and 21; whereas, the rare ROBs form through more variable mechanisms of double strand break repair such as nonhomologous end joining or homologous recombination.

Program Nr: 271 from 2002 ASHG Annual Meeting

**Meiotic synapsis in humans: bringing homologs together.** *P. Brown[1], L. Judis[1], E. Chan[1], A. Seftel[2], S. Schwartz[1,2], T. Hassold[1,2].* 1) Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Univ Hospitals of Cleveland, Cleveland, OH.

The intimate association of homologs, a process mediated by the meiosis-specific synaptonemal complex (SC), is a crucial initial step in meiosis. Studies of model organisms indicate that mutations in loci encoding SC proteins have profound adverse consequences on gametogenesis. Since meiosis is highly conserved it seems likely that abnormalities in SC formation also contribute to human sub- and infertility, and may have importance in the genesis of human aneuploidy. However, difficulties associated with acquisition of human meiotic tissue and the absence of imaging technologies have limited our ability to characterize human synapsis. Fortunately, advances in immunofluorescence/FISH methodology now allow us to simultaneously visualize the SC, SC-associated proteins, and individual chromosomes during all stages of prophase I. Thus, we have begun analyzing meiotic pairing and synapsis in fertile and infertile men. Initially, we are characterizing the normal situation, asking whether there are chromosome-specific patterns in the timing of synapsis, whether synapsis is initiated at one or multiple sites per chromosome, and whether specific chromosomal elements mediate chromosome pairing and/or synapsis in humans. To date, we have analyzed prophase I spermatocytes from four controls (i.e., males being treated for infertility, but for reasons unrelated to meiotic defects), using antibodies against SCP1 and SCP3 (to detect components of the SC), CREST antiserum (to detect kinetochores) and chromosome-specific FISH probes. Initial analyses of approximately 50 cells indicate that, in contrast to other organisms, centromeres appear not to promote pairing or synapsis. Telomeres are clearly important: most chromosomes initiate synapsis at two sites, one near the q-arm telomere and one near the p-arm telomere. However, acrocentric chromosomes are exceptions, as synapsis appears to proceed from the q-arm through the centromere to the p-arm. These initial results indicate remarkable chromosome-specific behavior in meiosis I; further characterization of these patterns may provide clues to the high incidence of aneuploidy in humans.

Program Nr: 272 from 2002 ASHG Annual Meeting

**Gaps in the synaptonemal complex are common in human males and alter meiotic exchange patterns.** *L. Judis*[1], *E. Chan*[1], *A. Seftel*[2], *S. Schwartz*[1,2], *T. Hassold*[1,2]. 1) Genetics, Case Western Reserve Univ, Cleveland OH; 2) University Hospitals of Cleveland, Cleveland OH.

To date there have been few studies utilizing modern cytological technologies to study meiotic recombination patterns in humans. We recently initiated immunofluorescence/FISH studies to characterize meiotic exchanges in fertile and infertile males, using antibodies against synaptonemal complex (SC) proteins and cross-over associated proteins (MLH1), CREST antiserum, and chromosome-specific FISH probes. We applied this strategy to examine pachytene stage spermatocytes from testicular biopsies of 20 males attending an infertility clinic, 14 individuals for infertility unrelated to meiotic defects (controls) and 6 for idiopathic infertility (cases). Results on over 2,000 meioses indicated significant variation in exchange rates among individuals and a small, but highly significant, reduction in exchanges in cases vs controls. Thus, we were interested in identifying sources of variation in either location or rate of exchanges. Unexpectedly, we found that one contributor involves perturbations in the SC. Specifically, the SC proteins SCP1 and SCP3 were often absent across sections of individual SCs; using FISH, we were able to assign 85% of the gaps to the heterochromatin of chromosomes 1, 9 or 16, with 9qh gaps accounting for 65% of events. Gaps did not alter overall rate of exchanges. However, they had a profound effect on their location: on gapped 9s, exchanges were significantly more likely to be distally located, both on 9p and on 9q; further, the larger the gaps, the more distally located the foci were on both arms. Thus, in humans as in other organisms, normal meiotic exchange patterns depend on fully-formed, tripartite SC. We also observed variation in the occurrence of gaps, as the proportion of cells with gaps ranged from 9-44% among individuals; unrelated to fertility status, age, or stage of pachytene. Possibly, the variation is due to individual differences in specific pericentromeric polymorphisms; we are now testing this idea. If this is the case, it would be an important first demonstration of a chromosome-specific effect on meiotic exchange patterns in humans.

Program Nr: 273 from 2002 ASHG Annual Meeting

**A mammalian model for human aneuploidy: sequence divergence causes meiotic nondisjunction in female mice.**
*K. Koehler, E. Millie, J. Cherry, S. Schrump, T. Hassold, P. Hunt.* Department of Genetics and Center for Human Genetics, Case Western Reserve University School of Medicine and the University Hospitals of Cleveland, Cleveland, OH.

Numerical chromosome abnormalities, present in an estimated 10-25% of all human conceptions, are the leading cause of pregnancy wastage in our species. These figures underscore the importance of identifying a mammalian model of human female nondisjunction, the source of most meiotic segregation errors. Prompted by the suggestion that the relatively outbred structure of the human population might contribute to the high observed levels of aneuploidy, we asked whether sequence divergence between homologous chromosomes in the female mouse could 1) mimic the altered recombination patterns observed in human trisomy-generating meioses and 2) increase nondisjunction from baseline levels observed in inbred strains of mouse (i.e., 1-2%) to rates approaching those identified in humans. We examined oocytes derived from F1 hybrids between C57BL/6 and *Mus spretus*, two discrete mouse species between which sequence divergence is about 1%. Pachytene preparations were processed using antibodies against SCP3 to identify synaptonemal complexes and MLH1 to identify late recombination nodules, the sites of crossing-over; MII preparations were analyzed using conventional cytogenetic methodology.

We observed striking differences in exchange patterns between the oocytes of the F1 and the parental animals. In the F1s, there was a significant decrease in recombination, in part due to an increase in nonexchange chromosomes; there was also an increase in altered recombination placements, including a shift toward distal-exchange events. Importantly, both of these phenomena are hallmarks of human nondisjunction. Furthermore, nondisjunction in the F1 oocytes was highly elevated, rising an order of magnitude from baseline, to over 10%. Thus, our initial results indicate that, by using the appropriate mouse strains, nondisjunction may be increased to a level comparable to that observed in humans, providing the first useful animal model of human female nondisjunction.

Program Nr: 274 from 2002 ASHG Annual Meeting

**CENP-A chromatin contains “euchromatic” histone modifications.** *B.A. Sullivan, A.D. Skora, H.D. Le, G.H. Karpen.* Mol & Cell Bio Lab, Salk Institute, La Jolla, CA.

Modifications of histone tails, termed the histone code, specify functional and structural chromatin domains in eukaryotes. Methylation of histone H3 at lysine 9 (H3K9) denotes heterochromatin, creating a binding site for HP1. It distinguishes centric repeats and other heterochromatic sites in yeast and the inactive X in mammals. Conversely, methylated H3 at lysine 4 (H3K4) is found in euchromatin, like many acetylated histones. Eukaryotic centromeres, located in repetitive DNA, are thought to be heterochromatic. Megabases of alpha satellite have been correlated with functional human centromeres. We found that only part of the alpha satellite array is associated with CENP-A, the centromeric histone required to recruit other kinetochore components. This raises the possibility that alpha satellite encodes other functions besides associating with CENP-A chromatin. Interspersed subdomains of both CENP-A and H3 are present within human centromeric chromatin. To determine if H3 in these subdomains is modified, we analyzed metaphases and extended chromatin fibers with antibodies to CENP-A and methylated and acetylated H3. Surprisingly, we found that methylated H3K9 was absent from the CENP-A domain, but present in the pericentric heterochromatin flanking the CENP-A domain. Interestingly, H3 within the CENP-A domain was methylated at lysine 4, a putative euchromatic modification. Acetylated H3K9 was largely excluded from CENP-A chromatin. Our findings indicate that kinetochore chromatin is not strictly heterochromatic, and instead displays “euchromatic” characteristics. We also provide direct evidence that alpha satellite DNA does indeed contribute to both heterochromatin and kinetochore chromatin formation within centromeric regions of human chromosomes. Human centromeres, like those in flies and fission yeast, are flanked by heterochromatin that is marked by methylated H3K9, an organization that may establish or protect CENP-A chromatin. Properties of centromeric chromatin, such as interspersion of H3 with CENP-A subdomains and modifications of H3 within the kinetochore and in flanking heterochromatin, may contribute to the 3-D structure of the kinetochore.

Program Nr: 275 from 2002 ASHG Annual Meeting

**Identification of a genomic hotspot and delineation of a mechanism for 9p terminal deletions.** *M. Eichenmiller[1], C.A. Crowe[2], L. Christ[1], D.L. Satinover[1], S. Schwartz[1]*. 1) Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH; 2) MetroHealth Medical Center, Cleveland, OH.

Over the past several years, molecular studies have delineated the mechanism of origin of a variety of chromosomal abnormalities. Specific repeats have been identified as leading to microdeletions, such as Prader-Willi and Smith-Magenis syndrome. More recently, it has been shown that AT-rich palindromes mediate the formation of the common t(11;22). However, little is still know about the formation of terminal deletions. In our study of 9p deletions we have examined over 85 structural rearrangements involving 9p. We have identified a 4.3Mb genomic hotspot in 9p22, in which we have constructed an STS/BAC contig consisting of 37 BACs and 34 STSs. We have identified 32 abnormalities in this region and localized each of the breakpoints between two BACs. Of those informative cases, eighty-five percent of these abnormalities were paternal in origin. A genomic analysis of the breakage region has revealed that this region has a lower GC content and higher frequency of repetitive sequences, such as SINES, LINES and LTRs, than expected. From this region, we have isolated five individual terminal deletions in somatic cell hybrids, allowing better elucidation of the mechanism of formation of these deletions. A series of PCR primers made from the genomic sequence surrounding the putative breakpoints helped to isolate the actual breakpoints to less than a 1Kb region in some cases. In the three cases where the breakpoint could be identified thus far, it has been isolated to a repetitive sequence, either a LINE or SINE element. This is an important study showing a detailed analysis of terminal deletions and based on these findings, we have gained insights concerning the mechanism of formation of terminal deletions: (1) we have identified an area of increased breakage in 9p22; (2) in the hotspot region there was an altered GC content and frequency of repetitive DNA compared to that normally seen; (3) breakpoints of 5 paternal terminal deletions have been isolated and 3 identified; and (4) these breakpoints were localized to highly repetitive regions involving either LINE or SINE elements.

Program Nr: 276 from 2002 ASHG Annual Meeting

**The linkage disequilibrium (LD) detected with multiallelic and biallelic markers in early and late settlement regions of Finland.** *T. Varilo[1], T. Paunio[1], A. Parker[3], M. Perola[1,2], J. Meyer[3], J.D. Terwilliger[4], L. Peltonen[1,2]*. 1) Dept Molec Med, NPHI, Helsinki, Finland; 2) Dept of Human Genetics, UCLA, LA, CA; 3) Millennium Pharmaceuticals Inc, Cambridge, MA; 4) Dept of Psychiatry and Columbia Genome Center, Columbia U, NY.

LD has been successful in the fine mapping of monogenic disease loci in isolates but its usefulness in the locus hunt for polygenic diseases has been challenged. We compared the extent of LD in three genealogically well characterized regional subpopulations of Finland: (i) the main population from early settlement (ES) descended 2,000-4,000 years ago, (ii) late settlement (LS), inhabited 15 generations ago, and (iii) a regional isolate (IS) founded by 39 families 12 generations ago. We genotyped 212 microsatellites over 62 cM, 45 SNPs over 0.43 cM and 23 SNPs over 0.38 cM on 1q. The genealogy of the ES (n=16), LS (n=54) and IS families (n=54) were defined carefully until early 1800s. Multiallelic markers exposed more LD in IS with a restricted number of founder ancestors than in LS and ES. In IS, 76% of pairs showed significant LD over the interval <0.5 cM, 30% over 0.5-7.5 cM, 12% over 7.5-12 cM, and 7% over 12 cM intervals; correspondingly for LS: 52%, 8%, 4% and 5%, and for ES: 19%, 5%, 4% and 5%, respectively. The LD did not only correlate with the distance but also with the information content of the markers. When only 5% of markers with the highest heterozygosity value were used (1,353 marker pairs with mean heterozygosity >0.79), the corresponding values for IS were: 97%, 60%, 17% and 9%. Opposite to wide LD-intervals detected with informative multiallelic markers, the SNPs detected LD only over intervals up 0.1 cM. Our data are in accord and exceed some earlier findings in this population by demonstrating that young expanded population isolates show LD detectable with multiallelic markers over wide genetic distances. Findings also emphasize the importance of detailed information of the population history and genealogy as well as the careful selection of markers for the genomewide LD-based studies aiming at identification of common disease genes.

Program Nr: 277 from 2002 ASHG Annual Meeting

**How to best use SNPs within a gene to test association?** *A.S. Jannot[1, 2], L. Essioux[2], M.G. Reese[2], F. Clerget-Darpoux[1]*. 1) Unite 535, INSERM, Kremlin Bicetre, France; 2) ValiGen, La Defense, France.

When multiple SNPs have been genotyped on a candidate gene, the haplotype can contain more information than each SNP taken separately (Verpillat, Bouley et al. 2001). Indeed, the model underlying the association of the gene to the trait can be complex involving a combination of intragenic SNPs. To optimally use the SNPs information, we propose a strategy called combination test that tests the association between the phenotype and all possible phased combinations of SNPs. We compare this strategy to two alternative strategies: the association test that considers each SNP separately and a multilocus genotype-based test that considers all the SNPs together. When two of the SNPs identified in the gene are functional with weak individual effects, the combination test is the only one able to detect the association with the phenotype. Interestingly, even in the case of a single functional SNP, the decrease of power when the combination test is used is weak compared to the strategy testing each SNP separately. As a consequence, the combination test gives a new impulse to association studies. We show, by an illustration on the association between lipid levels and some candidate genes that this strategy can help also in discovering which SNP or combination of SNPs give the susceptibility to the studied trait and merit the next level of biological study.

Program Nr: 278 from 2002 ASHG Annual Meeting

**An empirical estimate of the number of SNPs required for a whole genome association study.** *M.E. Zwick, D.J. Cutler, S. Lin, A. Chakravarti.* McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine.

Identifying the heritable genetic variation underlying human disease susceptibility is a central goal of human genetics. Whole-genome association studies using a high-density SNP haplotype map, in a case-control design, are one approach that may enable this research program. While theoretical predictions for the density of SNPs necessary for such a study have been available, empirical assessments are rare. Our resequencing of approximately 0.1% of the unique regions of forty human genomes with high-density probe arrays enables us to provide such an empirical estimate.

We report four main findings. First, the extent of linkage disequilibrium (LD), using both pairwise measures among segregating sites and 4Nc, varies by two orders of magnitude among genomic regions. Second, using X chromosome haploid data to generate diploids, we show that computational haplotype reconstruction of common SNPs (frequency>20%) using the PHASE algorithm can be more than 95% accurate over the 150 kb genomics regions that we resequenced. Third, employing a simple block definition ($D^1$>0.8 among all pairs of sites in a block) that describes the correlation among common SNPs, we observe that most sites are found in blocks (5% of SNPs are in singleton blocks) that are <10kb in size (average block size: 11kb). Finally, if we define blocks at $D^1$>0.8 and exclude singleton blocks, genotyping slightly more than 200,000 SNPs will recover roughly 50% of the haplotype heterozygosity, while genotyping 400,000 SNPs will recover greater than 80% of the haplotype heterozygosity. Including singleton blocks raises the number of SNPs required to be genotyped (350,000 SNPs for 50%; 525,000 SNPs for 80% of the haplotype heterozygosity). Because of the extensive genome-wide variation in LD, choosing an optimal set of SNPs will require prior knowledge of the patterns of LD and haplotypic variation from throughout the human genome.

Program Nr: 279 from 2002 ASHG Annual Meeting

**Haplotype block definitions and their relationship with population history, recombination rate, and SNP density: Simulation and data.** *N. Wang[1], JM. Akey[1], K. Zhang[2], R. Chakraborty[1], L. Jin[1]*. 1) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 2) Human Genetics Center, University of Texas-Houston, Houston, TX.

Recent studies suggest that haplotypes are arranged into discrete block-like structures throughout the human genome. Here we propose a conceptually intuitive and more rigorous haplotype block definition, which assumes no recombination within each block, but allows for recombination between blocks. This definition allows for less ambiguity, compared with other definitions based on linkage disequilibrium or haplotype diversity, and can be used to study the origin of haplotype block structure. To this end, we also investigate how various population genetics parameters affect haplotype block characteristics through coalescent-based simulations. As expected, we find that the average block size decreases with increasing recombination levels, populations of smaller effective size manifest larger haplotype blocks in comparison to larger populations, and sample size dramatically effects haplotype block identification. The density of the SNPs also significantly contributes to haplotype block identification and characteristics, and we show that the appropriate SNP density, best suited for haplotype block studies in human populations, is also dependent on recombination rate. Finally, we apply our novel haplotype block identification algorithm to published SNP data on chromosome 21 and demonstrate that recombinational hot spots are not necessary to generate haplotype block patterns.

Program Nr: 280 from 2002 ASHG Annual Meeting

**A Dynamic Programming Algorithm for Haplotype Block Partitioning and Its Application in Association Studies.** *K. Zhang, P. Calabrese, T. Chen, M. Deng, M. Nordborg, M. Waterman, F. Sun.* Program in Molecular and Computational Biology, Department of Biology, University of Southern California, Los Angeles, CA.

Recent studies showed a haplotype block structure for the human genome such that it can be divided into discrete blocks of limited haplotype diversity. A small fraction of SNPs (tag SNPs) can be used to distinguish a large fraction of the haplotypes in each block. These tag SNPs can be extremely useful for association studies in that it may not be necessary to genotype all the SNPs. We develop a dynamic programming algorithm to partition the haplotypes into blocks. The algorithm is guaranteed to find the haplotype blocks with the minimum number of tag SNPs required to account for most of the common haplotypes in each block. We apply this algorithm to the chromosome 21 data of Patil et al. [Science 294, 1719-1723]. Using the same criteria as in Patil et al., we identify a total of 3,582 tag SNPs and 2,575 blocks that are 21.5% and 37.7% smaller, respectively, than those identified using a greedy algorithm of Patil et al. When the tag SNPs instead of all the SNPs are used to reduce the genotyping effort in association studies, an important question is how much power is lost. We develop the following simulation strategy to quantitatively assess the power loss. First, case-parental or case-control samples are generated based on a disease model. Second, a small fraction of samples are selected to determine the haplotype blocks and the tag SNPs by our dynamic programming algorithm. Third, the statistical power of tests is evaluated based on three kinds of data: (1) all of the SNPs and the corresponding haplotypes; (2) the tag SNPs and the corresponding haplotypes; (3) the same number of randomly chosen SNPs as the number of tag SNPs and the corresponding haplotypes. We study the power of different association tests with a variety of disease models and block partitioning criteria. Our study indicates that the genotyping efforts can be significantly reduced by the tag SNPs without much loss of power. Depending on the specific block partitioning algorithm and the disease model, on average, when the identified tag SNPs are only 25% of all the SNPs, the power is reduced by only 9%.

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**A method to find and compare the strength of haplotype block boundaries and its application in populations with different settlement histories.** *M. Perola[1,2], M. Koivisto[3], T. Varilo[2], W. Hennah[2], J. Ekelund[2], M. Lugg[3], L. Peltonen[1,2], E. Ukkonen[3], H. Mannila[3]*. 1) Dept Human Genetics, UCLA Sch Medicine, Los Angeles, CA; 2) Dept Molecular Medicine, National Public Health Institute, Helsinki, Finland; 3) Dept Computer Science, University of Helsinki, Helsinki, Finland.

The recent data of haplotype blocks have left multiple uncertainties concerning block boundaries and their variation in different populations. The published analyses have used segmentation algorithms, with little indication of the strenght of the detected boundaries. We have developed a new method for finding haplotype blocks based on the use of the minimum description length principle (MDL). The method provides estimates on the strength of each boundary between blocks, making it easy to interpret the results, giving the investigator a tool to evaluate objectively the haplotype division of the locus in question. We have applied this method to the published data of Daly et al.(2001). The results are in relatively good agreement with the published results but also show distinct differences in predicted block boundaries. We also applied the method to SNP data on samples from three subpopulations from Finland, which are representative for the settlement history of Finland, inhabited by two periods of immigration 4000 and 2000 years ago. The early settlement sample (n=32 chromosomes) consisted of descendants of the early settlers (~100 generations) on the coastal areas of Finland, the late settlement sample (n=108 chr.) representing a younger (15-20 generations) population that has gone through a population bottleneck in 1500s and the third regional subisolate (n=108 chr.), founded by 40 families some 300 years ago, followed by a major expansion. A total of 68 SNPs were genotyped over 1Mb area on chromosome 1q. Total of seven, very similar haplotype blocks were identified in three populations. The identified blocks varied in size from 12kb to 361kb. As expected, the haplotype blocks do not differ in different subpopulations of Finland, most probably reflecting the limited set of original founder chromosomes shared by all analyzed populations.

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**HapScope: a software system for automated and visual analysis of functional haplotypes.** *J. Zhang, W. Rowe, K. Buetow*. Laboratory of Population Genet, National Cancer Institute, Gaithersburg, MD.

Identification of genetic variations attributed to disease susceptibility requires knowledge about functional polymorphism and population history of haploytpes. We have developed a software analysis package, HapScope, which includes a comprehensive analysis pipeline and a sophisticated visualization tool, for functional haplotype analysis. The HapScope analysis pipeline supports: a) computational haplotype construction with the EM or the Phase algorithm; b) SNP classification by protein coding change, homology to model organisms, or putative regulatory regions; c) haplotype block construction; d) minimum SNP subset selection by either a brute force or a greedy partition algorithm. The HapScope viewer displays genomic structure with haplotype information in an integrated environment, providing eight alternate views for assessing genetic and functional correlation. It has a user-friendly interface for: a) missing data management; b) subset SNP selection either by SNP functional classification, allele frequency or user manual selection; c) haplotype construction with subset SNP markers; d) incorporation of experimentally determined haplotypes with computational results; e) data export for additional experimental or computational genomic and genetic analysis. The results from HapScope compare closely with experimentally derived haplotypes for ApoE and LPL genes. We have successfully applied HapScope in a large-scale genotyping project for breast cancer study using the candidate genes approach and envision that HapScope's ability to incorporate functional analysis with population genetics will greatly facilitate current genetic disease research.

Program Nr: 283 from 2002 ASHG Annual Meeting

**Randomly Distributed Recombination May Generate Block-like Pattern of Linkage Disequilibrium: An Act of Genetic Drift.** *K. Zhang[1,2], J.M. Akey[1], N. Wang[1], M. Xiong[2], R. Chakraborty[1], L. Jin[1]*. 1) Center for Genome Information, University of Cincinnati, Cincinnati, OH; 2) Human Genetics Center, University of Texas, Houston, TX.

Genome-wide association studies have been proposed as a powerful and promising approach to identify genes underlying complex diseases. The feasibility of this study design has been questioned however, until recently, when discrete block-like pattern of linkage disequilibrium (LD) were found in the human genome, including the 5q31 region, the MHC II region, and the most part of chromosome 21. The existence of block-like patterns of LD raises a hope that whole genome association studies can be carried out with a reasonable cost by genotyping only a small fraction of SNPs that represents most haplotype diversity within the blocks. It has been hypothesized that the genome wide pattern of linkage disequilibrium is primarily dictated by the presence of recombination hotspots. We have studied the effects of different recombination models (hotspot model based on the MHC class II region; randomly distributed crossover model with low, medium and high recombination rate settings) on patterns of LD using computer simulation. Haplotype block is defined as a region with minimal pair-wise |D'| exceeding a certain threshold value (0.8), and block patterns were identified with a greedy algorithm. We found recombination hotspots dictate haplotype block structure if they exist and the local recombination rate is sufficiently high. However, we also observed the formation of haplotype blocks in models where recombination is randomly and evenly distributed. We demonstrate that, under this model, genetic drift alone can generate block-like patterns of LD similar to those observed in human populations. In conclusion, we caution that genetic drift may be an alternative mechanism, besides the hotspot model, that may be responsible for the genesis of block-like patterns of LD. Our finding highlights the necessity of reconstructing haplotype blocks in multiple ethnic groups in order to ensure the results are broadly applicable.

Program Nr: 284 from 2002 ASHG Annual Meeting

**Linkage disequilibrium maps: 'blocks' without haplotyping and application to disease mapping.** *A. Collins.* Division of Human Genetics, Univ Southampton, Southampton, England.

Linkage disequilibrium (LD) maps (Maniatis et al, 2002, Proc Natl Acad Sci USA 99(4), 2228-2233) are analogous to linkage maps in that, when locations of markers (in linkage disequilibrium units, LDUs) are plotted against their corresponding locations in kilobases, a series of plateaus and steps is revealed. In the linkage map the steps correspond to recombination hot spots and the plateaus to segments with suppressed recombination. The available evidence suggests that, despite the influence of evolutionary and other factors, the same pattern observed for the LD map also reflects recombination with the hot spots being remarkably narrow (~ 1-2kb in some regions). The plateaus represent LD blocks and correspond to regions of low haplotype diversity. There is a close correspondence between blocks revealed as runs of low haplotype diversity and LD maps constructed from pairwise association data using the LDMAP program (http://cedar.genetics.soton.ac.uk/public_html/helpld.html). The latter, however, does not require the derivation of haplotypes but models the decline of allelic association in each map interval using SNP genotype data and expresses this as an additive map distance. Aside from the appeal of (unphased) diplotype analysis over haplotypes the LD maps define marker spacing for disease mapping in that, to ensure coverage of a region, markers should be spaced evenly on the LD map. This is in contrast to the kilobase map which requires higher and lower marker densities for coverage of 'step' and 'block' regions respectively. It is also possible that, even given optimal spacing on both scales, disease genes will be more precisely localised on the LD map. Results from simulated and real data will reveal more about the properties of this novel form of genome map.

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**SNP ascertainment is a crucial factor in determining LD block structure.** *A. Morris, R. Lawrence, L. Cardon.*
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There has been much recent interest in the extent and apparent block structure of linkage disequilibrium (LD) across the genome. Within blocks, little historical recombination has occurred, with only a few common haplotypes observed. We have investigated the distribution of LD blocks across chromosome 19 using more than 1800 SNPs, randomly ascertained through dbSNP, in 80 founder chromosomes from the CEPH reference families. A block is defined to consist of at least 3 SNPs, with a stringent threshold of D'>0.9 for each pair. The distribution of lengths is highly skewed, with a median of 20kb, 28% of blocks with length <10kb, and a maximum length of 625kb.

We present simulations to illustrate the effects of recombination rate, inter-SNP distance and SNP ascertainment on the distribution of block length. For each simulation, the ancestry of 1000 regions of length »1cM are each represented by means of an ancestral recombination graph, generated for a sample of 80 chromosomes under the shattered coalescent with recombination.

The results of the simulation study clearly demonstrate that the most important factor in predicating block length across chromosome 19 is SNP ascertainment, here modelled to reflect the observed uniform distribution of minor allele frequency. Bias in SNP ascertainment in favour of common minor alleles increases the relative frequency of short blocks. Common alleles are expected to be more ancient than relatively rare recent mutations, increasing the probability that sufficient historical recombination events have occurred to break down LD in a block.

The block structure of LD has motivated the concept of a haplotype map of the human genome, expected to aid association mapping of disease genes. Our results suggest caution. SNP ascertainment is a crucial determinant of block structure, and must be taken into account, along with density of coverage, in order to complete the construction of such a map.