Program Nr: 1 from the 2000 ASHG Annual Meeting

Mutations in the EGF-CFC gene, *CRYPTIC*, cause human Left-Right axis abnormalities and Transposition of the Great Arteries. *R.N. Bamford[1], J. dela Cruz[1], E. Roessler[1], U. Saplakoglu[2], R. Burdine[3], E. Goldmuntz[4], M. Shen[2], A. Schier[3], B. Casey[5], M. Muenke[1]*. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Robert Wood Johnson Medical School, Piscataway, NJ; 3) Skirball Institute, NYU, NY; 4) The Children's Hospital of Philadelphia, PA; 5) Baylor College of Medicine, Houston, TX.

Studies in zebrafish and mice demonstrate that the EGF-CFC proteins (oep/Cripto/Cryptic) play critical roles in the establishment of left-right (L-R) and anterior-posterior (A-P) axis formation as essential co-factors for cyclops/squint/Nodal proteins. Here we investigated the potential genetic role of *CRYPTIC* in human laterality disorders such as, *situs inversus, situs ambiguus* and isomerism of normally asymmetric organs. Although Transposition of the Great Arteries (TGA) is often seen in patients manifesting other *situs* complications, isolated TGA is felt to represent a unique disorder. Therefore, we also screened TGA patients for *CRYPTIC* mutations to determine if this disorder can be placed within the laterality spectrum. Although the *CRIPTO* gene had been characterized in humans, the *CRYPTIC* gene had not been analyzed. We cloned and characterized the human *CRYPTIC* gene, its cDNA, and performed mutational studies on genomic DNA from laterality and TGA patients. We have identified three missense and one frameshift mutation in the *CRYPTIC* gene of patients with laterality defects. In TGA patients, one unique splice-donor mutation has been identified, however, two mutations originally identified in laterality patients were also seen in two TGA patients. These nucleotide changes were not detectable in over 200 chromosomes from normal controls. Phenotypic rescue studies utilizing *oep* zebrafish mutants and cellular localization studies confirm the significance of these mutations in functional studies. In summary, our results demonstrae a role for *CRYPTIC* in the pathogenesis of human laterality defects and suggest that TGA can be included within the spectrum of laterality disorders.

Program Nr: 2 from the 2000 ASHG Annual Meeting

Genome-wide comparison of human genetic and physical maps. *A. Yu[1], C. Zhao[1], K.W. Broman[2], W. Jang[3], A.J. Mungall[4], I. Dunham[4], J.L. Weber[1]*. 1) Center for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI; 2) Department of Biostatistics, Johns Hopkins University, Baltimore, MD; 3) National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; 4) The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK.

Completion of draft and near complete human chromosomal DNA sequences permits a genome-wide analysis of the relationship between genetic and physical map distances. Using computer searches we have identified BAC sequences which encompass about 70% of the 8000 short tandem repeat polymorphisms (STRPs) on our most recent comprehensive genetic maps (Broman et al. Am. J. Hum. Genet. 63:861-869, 1998). Reasons for the 30% failure are not completely understood, but likely involve absence of the appropriate BAC sequence from the public database and the presence of interspersed repeats within the STR sequence. At the time of abstract submission (June 2000), we have obtained reliable physical distances in nucleotides between STRPs for three chromosomes: 6, 21 and 22. Physical distances for the remaining chromosomes are likely to become available in the near future. Comparison of the genetic and physical maps helps identify errors in the maps, regions of high and low recombination, and even putative inversion polymorphisms. For chromosomes 6, 21, and 22, marker orders agreed well between the genetic and physical maps. Only four STRPs appeared to be positioned with large error. Overall recombination rates (sex-average) for the three chromosomes ranged from 1.1 to 1.9 and averaged 1.24 cM/mb. For many chromosomal regions, female and male recombination rates changed in parallel, but for other regions there were large gender differences. Regions with particularly low recombination, near 0 cM/mb and spanning 6 - 12 mb, were found on all three chromosomes. Genes within these recombination deserts may be particularly amenable to mapping by association. Regions with particularly high average recombination, up to about 6 cM/mb were also identified. It is unknown if these recombination jungles have uniformly high levels of recombination or are in turn comprised of shorter regions of high and low recombination.

Program Nr: 3 from the 2000 ASHG Annual Meeting

Tandem mass spectrometry newborn screening for medium-chain acyl-CoA dehydrogenase deficiency in North Carolina. *S.E. McCandless[1], J. Muenzer[1], S.H. Chaing[2], S.D. Weavil[2], E.G. Moore[2], D.M. Frazier[1]*. 1) Department of Pediatrics, Univ North Carolina, Chapel Hill, NC; 2) Division of Public Health, Raleigh, NC.

In 8/97 the state of North Carolina (NC) expanded its newborn screening program by addition of tandem mass spectrometry (TMS) to detect a variety of metabolic disorders, including medium-chain acyl-CoA dehydrogenase deficiency (MCADD). From 8/97 to 4/99 the tests were performed by Neo Gen Screening, Inc. (Pittsburgh, PA). Since 4/99 screening has been performed by the NC State Newborn Screening Laboratory. As of 6/00 327,031 infants from NC (71% Caucasian) had been screened. Abnormal follow-up plasma acylcarnitine profile and/or urine organic acid analysis, or documentation of homozygosity for the A985G mutation confirmed suspected MCADD. To date, 24 asymptomatic infants with MCADD have been identified by TMS screening. Of these, 23 were Caucasian, giving an incidence of MCADD of 1:10,095 in that population. All of the Caucasian children tested had at least one copy of the common A985G mutation with 70% (14/20) homozygous. The one affected African-American infant had no copies of the common mutation. Confirmatory testing for suspected MCADD was striking. A985G homozygotes had a mean C8-acylcarnitine of 3.46 mM (nl<0.25) and mean C8/C10 ratio of 14.2 (nl=0.9), while MCADD infants with only one copy of A985G had a mean C8 of 1.10 mM and a mean C8/C10 ratio of 3.2. All infants were treated with carnitine and were maintained on breast milk or regular infant formula with no fat restriction in the first year of life. Parents were instructed to avoid prolonged fasting and to use glucometers to monitor blood glucose when they were concerned about the child's oral intake or clinical status. During 36.5 patient years of follow-up, 2 patients have been admitted a total of 4 times for IV glucose due to inadequate oral intake of fluids. There have been no deaths, no significant hypoglycemia, and no seizures in MCADD children identified by TMS screening. All children are developmentally normal so far. Our initial experience demonstrates that newborn screening by TMS, with careful follow-up, can prevent most, if not all, deaths and serious sequelae of MCADD in the first few years of life.

Program Nr: 4 from the 2000 ASHG Annual Meeting

Htra2-beta1 restores full-length SMN2 expression by activating an exonic splicing enhancer; An exciting target for therapy in spinal muscular atrophy. *B. Wirth[1], Y. Hofmann[1], C.L. Lorson[2], S. Stamm[3], E.J. Androphy[2]*. 1) Dept Molecular Genetics, Inst Human Genetics, Bonn, Germany; 2) Dermatology, New England Medical Center and Tufts University School of Medicine, Boston, Massachusetts, USA; 3) Max-Planck-Institute of Neurobiology, Martinsried, Germany.

Spinal muscular atrophy (SMA), the second most frequent autosomal recessive disorder in human is a motor neuron disease that results from loss of functional survival motor neuron (SMN1) alleles. A nearly identical copy of the gene, SMN2, fails to provide protection from SMA due to a single translationally silent nucleotide difference in exon 7. This likely disrupts an exonic splicing enhancer (ESE) and causes exon 7 skipping leading to abundant production of a shorter isoform, SMN2delta7. The truncated transcript encodes a less stable protein with reduced self-oligomerization activity that fails to compensate for the loss of SMN1. This report describes the identification of an in vivo regulator of SMN mRNA processing. Htra2-beta1, an SR-like, non-essential splicing factor and ortholog of Drosophila melanogaster transformer-2, promoted the inclusion of SMN exon 7 and stimulated full-length SMN2 expression to almost 90%. Htra2-beta1 specifically functioned through and bound an AG-rich ESE in SMN exon 7. This effect is not species specific as expression of Htra2-beta1 in human or mouse cells carrying an SMN2 minigene dramatically increases production of full-length SMN2. This demonstrates that SMN2 mRNA processing can be modulated in vivo. Since all SMA patients retain at least one SMN2 copy, the prevention of exon 7 skipping by upregulating the full-length product of SMN2 by Htra2-beta1 represents an exciting target for SMA therapy.

Program Nr: 5 from the 2000 ASHG Annual Meeting

Variability and Pathogenesis of DiGeorge Syndrome in Mice. *E.A. Lindsay, F. Vitelli, T. Pramparo, T. Huynh, A. Baldini.* Pediatrics - Cardiology, Baylor Col Medicine, Houston, TX.

DiGeorge syndrome (DGS) is an archetypal cranial/cardiac neurocristopathy that includes aortic arch and outflow tract defects, and aplasia/hypoplasia of the thymus and parathyroids. DGS is caused by a ~3 Mb heterozygous deletion of 22q11.2 (*del22q11*). We have generated a mouse model of *del22q11* that carries a chromosomal deletion (*Df1*) that eliminates most of the murine homologs of genes deleted in DGS. In a C57Bl6x129SvEv, mixed genetic background, *Df1* causes CHD but not thymic or parathyroid defects. This may be because the gene(s) responsible for these findings lie outside *Df1*, or because phenotypic expression depends upon genetic modifiers elsewhere in the genome. To address these two possibilities we have a) generated additional chromosomal deletions including genes not deleted in *Df1* but deleted in DGS, and b) analyzed the *Df1*/+ phenotype in different mouse strains. Results suggest that deletion of genes outside Df1 is irrelevant to the DGS neurocristopathy. In contrast, breeding the *Df1* deletion into a different genetic background resulted in the expression of at least one additional DGS finding, namely thymic defects. The parathyroid phenotype is being ascertained. Surprisingly, in this inbred strain, the penetrance of cardiovascular defects was lower than in the mixed strain suggesting that different genetic modifiers may affect the expression of different aspects of the DGS phenotype. These findings indicate that the *Df1*/+ mouse may be a complete model of DGS, and offer the opportunity to identify genetic modifiers potentially underlying the striking phenotypic variability of the human syndrome. To understand the role of neural crest in the pathogenesis of the *Df1*/+ phenotype, morphological and molecular phenotypic analyses were carried out in *Df1*/+ embryos. Results suggest that *Df1* does not affect neural crest cell migration, proliferation or apoptosis, but causes defective differentiation of neural crest-derived mesenchymal cells. We hypothesize that there is a failure of tissue interaction pathways and propose a model illustrating how haploinsufficiency of a gene within *Df1* may lead to the different findings of the DGS neurocristopathy.

Program Nr: 6 from the 2000 ASHG Annual Meeting

First clinical test with recombinant human a**-glucosidase from rabbit milk shows therapeutic effect in Pompe patients.** *H. van den Hout[1], A. Reuser[2], A. Vulto[1], W.F. Arts[1], A. Cromme-Dijkhuis[1], W. Hop[2], A. van der Ploeg[1]*. 1) Depts. of Pediatrics, Hospital Pharmacy and Child Neurology, Sophia Children's Hospital, Rotterdam; 2) Depts. of Clinical Genetics and Biostatistics and Epidimiology, Erasmus University Rotterdam, The Netherlands.

Pompe's disease or Glycogen storage disease type II (GSD II) is a muscular disorder caused by an inherited deficiency of lysosomal a-glucosidase and concomitant storage of glycogen. The infantile form presents with a characteristic hypertrophic cardiomyopathy and generalized muscle weakness. Milestones like rolling over, sitting and standing are not achieved. Patients die within one year of age. Late onset forms are more slowly progressive and present as a proximal myopathy with involvement of respiratory muscles. Until now no treatment was available. Our research has focussed on the development of enzyme replacement therapy for Pompe's disease by testing different methods of recombinant human a-glucosidase production and has led to large-scale production of human recombinant a-glucosidase in milk of transgenic rabbits for therapeutic purpose. The efficacy of the enzyme was preclinically shown in a knockout mouse model for Pompe's disease. A phase I study was preformed in healthy volunteers. On base of these findings, a single center pilot study with the recombinant human a-glucosidase from rabbit milk was started in the Sophia Children's Hospital; 4 infantile and 3 juvenile patients were included. The infantile patients are currently treated for over a year. All patients had a severe deficiency of a-glucosidase and a characteristic cardiomyopathy. Ages ranged from 2.5 to 8 months at start. The dose was 15 to 20 mg/kg/week and later increased to 40 mg/kg. a-glucosidase activities normalized in skeletal muscle in all patients, and tissue morphology improved. By 36 weeks of treatment cardiac size had decreased significantly to less than 30 % of baseline. Motor function improved, respiratory insufficiency could be prevented when treatment started at a young age. In conclusion recombinant a-glucosidase from transgenic rabbit milk has therapeutic effects in patients with Pompe's disease.

Program Nr: 7 from the 2000 ASHG Annual Meeting

A strong candidate prostate cancer predisposition gene at chromosome 17p. *S.V Tavtigian[1], J. Simard[2], F. Labrie[2], M.H. Skolnick[1], S.L. Neuhausen[3], J. Rommens[4], L.A. Cannon Albright[3,5]*. 1) Myriad Genetics, Inc., Salt Lake City, UT; 2) Oncology and Molecular Endocrinology Research Center, Laval University Hospital and Laval University, Quebec City, Canada; 3) Genetic Epidemiology, Department of Medical Informatics, University of Utah School of Medicine, Salt Lake City, Ut; 4) Department of Genetics, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; 5) Genetic Research, Intermountain Health Care, LDS Hospital, Salt Lake City, Ut.

The late age at diagnosis of prostate cancer, presence of phenocopies within high-risk pedigrees, and genetic complexity of the trait, all contribute to the difficulty of identification of prostate cancer predisposition genes. Linkage analysis of a genome-wide scan of large, high risk Utah pedigrees provided evidence for a prostate cancer predisposition locus on chromosome 17p. Positional cloning and mutation screening within the refined interval identified a gene, HPC2/ELAC2, harboring mutations that segregate with prostate cancer in two high-risk pedigrees. In addition, two common missense changes in the gene were found to be associated with a diagnosis of prostate cancer. Survival analysis of cases carrying one of these common missense variants, Ala541Thr, showed a significant decrease in length of survival compared to non-carrying cases in the pedigrees. HPC2/ELAC2 is a member of an uncharacterized gene family conserved among the multicellular eukaryotes. The gene is predicted to encode a metal-dependent hydrolase domain that is conserved among the eukaryotes, archaebacteria, and eubacteria, and bears striking amino acid sequence similarity to domains present in two better understood protein families, namely the PSO2 (SNM1) DNA interstrand crosslink repair proteins and the 73 kDa subunit of mRNA 3' end cleavage and polyadenylation specificity factor (CPSF73).

Program Nr: 8 from the 2000 ASHG Annual Meeting

Incontinentia Pigmenti results from a common mutation in the NEMO gene and affects cellular signal transduction through the NF-kB pathway. *S. Aradhya[1], H. Woffendin[2], T. Esposito[3], A. Smahi[4], G. Courtois[5], T. Jakins[2], T. Bardaro[3], A. Ciccodicola[3], R.A. Lewis[1], D.L. Nelson[1], S.J. Kenwrick[2], M. D'Urso[3], A. Israël[5], A. Munnich[4].*
1) Baylor College of Medicine, Houston, TX, USA; 2) University of Cambridge, Cambridge, UK; 3) International Institute of Genetics and Biophysics, Naples, Italy; 4) Hopital Necker-Enfants Malades, Paris, France; 5) Pasteur Institute, Paris, France.

X-linked dominant Incontinentia Pigmenti (IP) is lethal in males and affects numerous organ systems in females. Skin defects involve blistering lesions that resolve as areas of decreased pigmentation. Tooth, eye, brain and nail abnormalities are also common. We recently found mutations in the NF-kB essential modulator (NEMO) gene in patients with IP (Nature 405:466, 2000). NEMO is essential for activation of the NF-kB signal transduction cascade in response to several stimuli including IL-1 and TNF. A common deletion mutation is found in IP patients. It removes exons 4-10 due to an aberrant recombination between identical LTR-like sequences present in the third intron of NEMO and distal to the final exon. This event occurs primarily in the male germline through intrachromosomal exchange. A survey of 200 probands in familial and sporadic IP cases has identified mutations in 150 individuals. Of these, the majority (135/150, or 90%) carry the deletion mutation. The high frequency of this mutation simplifies DNA diagnosis. The remaining mutations comprise loss of function and missense mutations. Many of these are found in the final exon of the gene. NEMO activity assays reveal partial function for some alleles, and are consistent with phenotypic aspects of the disorder in some families. Of particular note are rare male IP patients who appear to have a less severe class of mutation, and whose phenotypes suggest roles for NEMO in angiogenesis, bone development and immune function. Mutational analysis of NEMO is complicated by the presence of a partial pseudogene located ~100 kb distal in Xq28. Sequence analysis of the pseudogene reveals >99.9% identity with the NEMO gene spanning exons 3-10, and complete identity within the duplicated exons.

Program Nr: 9 from the 2000 ASHG Annual Meeting

Pseudoxanthoma Elasticum: Mutations in the MRP6 Gene Encoding A Transmembrane ABC Transporter. *F. Ringpfeil[1], A. Nakano[1], M.G. Lebwohl[2], L. Pulkkinen[1], J. Uitto[1]*. 1) Dermatology Cutaneous Biol, Thomas Jefferson Univ, Philadelphia, PA; 2) Mt. Sinai School of Medicine, New York, NY.

Pseudoxanthoma elasticum (PXE) is a heritable systemic connective tissue disorder affecting the skin, the eyes and the cardiovascular system. A characteristic histopathological finding is progressive calcification of elastic fibers in the affected organs, leading to considerable morbidity and mortality. The genetic basis of PXE has remained unknown, but the gene has recently been mapped to an ~500 kb interval on chromosome 16p13.1. One of the candidate genes within this interval is MRP6, an ABC transporter, which is here shown to harbor mutations in PXE. We first developed a mutation detection strategy based on PCR amplification of MRP6 coding segments using total genomic DNA as template, followed by heteroduplex scanning of the PCR products and automated nucleotide sequencing. Under the optimized conditions, all 31 exons and the flanking intronic sequences of MRP6 could be amplified, and heteroduplex analysis was able to identify both pathogenetic and neutral sequence variants. A total of 21 mutant MRP6 alleles were disclosed in the 16 probands with PXE. These genetic lesions consisted of either single basepair substitutions resulting in missense, nonsense or splice site mutations, a single-basepair deletion, or large deletions resulting in allelic loss of the MRP6 locus. Examination of clinically unaffected family members in multiplex families identified heterozygous carriers, consistent with an autosomal recessive inheritance pattern. Collectively, identification of mutations in the MRP6 gene provides the basis to examine the pathomechanisms of PXE, and allows DNA-based presymptomatic diagnosis and carrier detection, as well as prenatal testing and preimplantation genetic diagnosis in families with a history of this disease.

Program Nr: 10 from the 2000 ASHG Annual Meeting

Pseudoxanthoma Elasticum : Identification of Mutations in the ABCC6 Gene. *D.P. Germain, J. Perdu, V. Remones, X. Jeunemaitre.* Dept Genetics, Hosp European Georges Pompidou, Paris, France.

Pseudoxanthoma elasticum (PXE) is an inherited disorder of connective tissue in which the elastic fibers of the skin, eyes, and cardiovascular system slowly become calcified, causing a spectrum of disease involving these 3 organ systems, with variable phenotypic expression. PXE is usually found as a sporadic disorder, but examples of both autosomal recessive (OMIM 264800) and autosomal dominant (OMIM 177850) forms have been documented. The PXE locus has been mapped to chromosome 16p13.1, and was recently further refined to a 500 kb-region, containing 4 candidate genes. We analysed by SSCP and direct sequencing the whole coding region of the pM5, UNK, and MRP1 genes, candidates on the basis of their genetic localization, in 7 unrelated PXE families, but failed to find evidence of disease-causing defects. 6 non-synonymous variants were found in the pM5 gene, and 2 in the MRP1 gene. An analysis of 50 control alleles showed that all variants were neutral polymorphisms. We thus considered ABCC6 (also known as MRP6), another candidate gene mapping to 16p13.1, which we found to be expressed at a low level in cultured skin fibroblasts from patients and controls. The ABCC6 gene was therefore investigated at the genomic level in the 7 PXE pedigree. Direct sequencing of all 31 exons resulted in the identification of several pathogenetic mutations associated with all genetic forms of PXE. A C>T transition was found at position 3421 in the ABCC6 cDNA sequence in 3 unrelated families. This mutation alters the codon for arginine 1141 to a stop codon (R1141X). Other identified mutations include frameshift and missense mutations. We also corrected minor errors in the original sequence, and identified a G to A transition at position 3803 in the ABCC6 cDNA sequence (R1268Q). This change should not be wrongly considered as disease-causing, since we found the Q1268 allele at a high frequency (0.2) in a control population (n=62), and at the homozygous state in 3 healthy volunteers. Our results indicate that the MRP6 gene, which encodes a 1503 amino-acids membrane protein, member of subfamily C of the ATP binding cassette (ABC) transporters, is mutated in PXE patients.

Program Nr: 11 from the 2000 ASHG Annual Meeting

FGFR3 mutations associated with acanthosis nigricans increase Bcl-2 expression and resistance to induced apoptosis in keratinocytes. *G.A. Bellus, D.P. Kelly, D.A. Norris.* Dept Dermatology, Univ. Colorado School of Medicine, Denver, CO.

Acanthosis nigricans (AN) is a dermatological finding associated with constituitively activating FGFR3 mutations that cause a Crouzon syndrome variant (A391E) and SADDAN syndrome (K650M). Activation of growth factor receptor signaling pathways (IGF-1R & EGFR) have been implicated in other forms of AN and are known to increase anti-apoptotic defenses in keratinocytes. We hypothesize that FGFR3 mutations may cause epidermal acanthosis by interfering with programmed cell death pathways that function normally in keratinocyte differentiation.

Skin samples of AN were obtained from patients with A391E and K650M mutations during scheduled surgical procedures. Keratinocytes were cultured from part of the sample and the rest was used for immunohistochemical analysis. Keratinocyte cultures were subjected to 3 apoptotic triggers (UV-B irradiation, cisplatinum & beavericin) and survival and apoptosis were determined by MTT assay and EtBr/AO staining. Immunohistochemical analysis of frozen AN skin sections from K650M and A391E patients revealed strong expression of the anti-apoptotic protein Bcl-2 in the upper differentiated epidermal layers while Bcl-2 expression was confined to the basal layer in control skin. Western blot analysis revealed that cultured K650M and A391E keratinocytes expressed about 4 times as much Bcl-2 as control keratinocytes. K650M keratinocytes were highly resistant to all 3 apoptotic triggers in comparison to control keratinocytes. A391E keratinocytes were resistant to UV-B and cisplatinin but sensitive to beauvericin.

We conclude that thickening of the epidermal layer in FGFR3 related AN may result directly from prolonged keratinocyte survival in the upper differentiated layers of the epidermis. These results are consistent with the oncogenic role of FGFR3 mutations described in multiple myeloma, bladder and cervix carcinomas but contradict other studies in chondrocytes where FGFR3 mutations lead to cell cycle arrest and increased susceptibility to apoptosis. Together, all of these results suggest that the downstream effects of FGFR3 signaling may be highly tissue specific.

Program Nr: 12 from the 2000 ASHG Annual Meeting

Autosomal dominant hypophosphatemic rickets (ADHR) is caused by mutations in a gene encoding a novel member of the fibroblast growth factor family (FGF-21). *B. Lorenz-Depiereux[1], K.E. White[2], W.E. Evans[2], M.C. Speer[3], J.L.H. O'Riordan[4], T. Meitinger[1], M.J. Econs[2], T.M. Strom[1].* 1) Medizinische Genetik, Ludwig-Maximilians-Universität, München, Germany; 2) Indiana University School of Medicine, Indianapolis, IN; 3) Duke University Medical School, Durham, NC; 4) University College London, London, UK.

ADHR is a renal phosphate wasting disorder characterized by low serum phosphate concentrations, rickets, osteomalacia, lower extremity deformities, short stature, bone pain, and dental abscesses. The disease has previously been mapped to an 18 cM interval between D12S100 and D12S397 in 12p13. We used a positional cloning approach to identify the ADHR gene. Fine mapping in two families with LOD scores of 7.68 and 1.1 at D12S1612 provisionally narrowed down the critical interval to 1.5 MB between D12S1685 and D12S1594. BAC mapping data and genomic sequences were available from the AECOM and the BCM, respectively. An annotation of genomic sequences between D12S1685 and D12S1623 revealed 37 genes, 13 of which were novel. The complete CDS of the novel genes were obtained by RT-PCR, RACE and/or sequencing of IMAGE clones. One of the novel genes shared homology with the fibroblast growth factor family, and was named FGF-21. FGF-21 is comprised of 3 exons, spanning app. 10 kb of genomic sequence. The longest FGF-21 RT-PCR product we obtained was 1612 bp and contained an ORF of 251 amino acids. Northern analysis showed the presence of 3.0 and 1.3 kb transcripts in RNA from the mylogenous leukemia cell line K562. The murine ortholog also has an ORF of 251 amino acids that shares 73% identity with human FGF-21. Mutation screening was performed in 4 families with male to male transmission. Direct sequencing of FGF-21 exons revealed 3 missense changes affecting 2 arginines, which lie 3 amino acids apart. The FGF-21 mutations segregated with the disorder in each ADHR family and were not detected in 450-800 control alleles. In summary, we have identified a novel factor, FGF-21, that when mutated, is responsible for the phosphate wasting disorder ADHR. Therefore, FGF-21 likely plays an important role in the normal regulation of human phosphate homeostasis.

Program Nr: 13 from the 2000 ASHG Annual Meeting

Bone density defects in sclerosteosis are the result of mutations in a novel, cysteine knot-containing secreted factor. *M.E. Brunkow[1], J. Van Ness[1], B.W. Paeper[1], B. Kovacevich[1], P.J. Sabo[1], S. Proll[1], J.E. Skonier[1], L. Zhao[1], J. Gardner[2], P. Beighton[2], P. Tacconi[3], D.J. Galas[1], J.T. Mulligan[1]*. 1) Celltech Chiroscience, Inc, Bothell, WA; 2) Univ. of Cape Town Medical School, Observatory, South Africa; 3) Istituto di Neurologia, Università di Cagliari, Italy.

The severe, sclerosing bone dysplasia, sclerosteosis (OMIM 269500), is characterized by generalized skeletal overgrowth leading most notably to gigantism, massive thickening of the skull and mandible, and ultimately to facial palsy, deafness, optic atrophy and eventually death due to elevated intracranial pressure. This rare disease has been classified as craniotubular hyperostoses, distinguishable from osteopetrosis by the bony overgrowth of skull and mandible, thickened cortices of the long bones and increased density of vertebral pedicles, ribs and pelvis. Based on analysis of serum markers of bone remodeling, it has been thought to be a result of osteoblast dysfunction.

The gene for sclerosteosis was previously localized to chromosome 17q12-21. We undertook physical mapping of this region, along with fine genetic mapping in 22 families from the Afrikaner community in South Africa. The disease locus was mapped to a ~3 cM region between D17S1787 and D17S930. A BAC contig of ~3 Mb was constructed across this interval, and extensive sequencing of clones comprising the minimal tiling path resulted in the identification of ~100 transcription units. For mutation detection, we used PCR amplification and direct sequencing of known and predicted exons out of patient genomic DNA.

We identified a single transcription unit with a disease-specific polymorphism resulting in translation termination immediately after an hypothetical signal sequence. This novel gene, which we call BEER (Bone-Expressed Equilibrium Regulator), is comprised of two exons and encodes a protein predicted to be a member of the large family of cysteine-knot-containing secreted factors. An additional, unrelated sclerosteosis patient was identified and shown to carry a splicing mutation in the BEER gene, thus supporting its identity as the sclerosteosis gene.

Program Nr: 14 from the 2000 ASHG Annual Meeting

Mutations in the human *Delta* homologue, *DLL3*, a *Notch* signaling pathway gene, disrupt somite boundary formation in spondylocostal dysostosis, which demonstrates both clinical and genetic heterogeneity. *P.D. Turnpenny[1,2], M.P. Bulman[2], K. Kusumi[3], T.M. Frayling[2], J. Duncan[2], C. McKeown[4], C. Garrett[5], R. Krumlauf[3], A.T. Hattersley[2], S. Ellard[2]*. 1) Clinical Genetics, Royal Devon & Exeter Hospital, Exeter, England; 2) University of Exeter, England; 3) National Institute for Medical Research, London, England; 4) Clinical Genetics Unit, Birmingham, England; 5) Kennedy Galton Centre, Harrow, Middlesex, England.

The spondylocostal dysostoses (SD) are a heterogeneous group of short trunk, short stature syndromes of varying severity due to developmental defects of the axial skeleton, characterised by extensive vertebral malsegmentation, rib fusions/deletions, and non-progressive kyphoscoliosis. We recruited families demonstrating autosomal recessive (AR) inheritance and by homozygosity linkage mapping identified a critical interval narrowed to 7.8 cM on chromosome 19q13.1-q13.3 (*Am J Hum Genet 1999;65:172-185*). This interval shares syntenic conservation with a region on mouse chromosome 7 containing the *Delta-like3 (Dll3)* gene, which encodes a transmembrane ligand for the Notch receptor. *Dll3* is mutated in the X-ray induced mouse mutant pudgy (*pu*), whose axial skeletal phenotype is similar to SD. We therefore cloned and sequenced human *DLL3* to evaluate it as a candidate gene for AR SD and identified mutations in three affected families demonstrating 19q13 linkage and sharing a very similar radiological phenotype. Two mutations predict protein truncations within conserved extracellular domains and the third is a missense mutation in a highly conserved glycine residue of the fifth epidermal growth factor repeat, suggesting an important functional role for this domain. This is the first cloning of a human *Delta* homologue. Non-linkage to 19q in families with both similar and dissimilar radiological phenotypes, including Jarcho-Levin syndrome and phenotypes with additional syndromic features, confirms genetic heterogeneity in the spondylocostal dysostoses.

Program Nr: 15 from the 2000 ASHG Annual Meeting

A syndrome of X-linked thrombocytopenia and thalassemia is due to a mutation in the transcription factor GATA-1. *W.H. Raskind[1], C. Yu[2], K.K. Niakan[1], M. Matsushita[1], G. Stamatoyannopoulos[1], S.H. Orkin[2,3].* 1) Department of Medicine, University of Washington, Seattle, WA; 2) Department of Pediatrics and; 3) Howard Hughes Medical Institute, Harvard Medical School, Boston, MA.

X-linked thrombocytopenia associated with thalassemia (XLTT) is a rare disorder characterized by moderate thrombocytopenia, normal platelet morphology, platelet dysfunction, splenomegaly, reticulocytosis, and unbalanced hemoglobin chain synthesis resembling b-thalassemia trait. In a four generation family, we mapped XLTT to chromosome Xp11-12 and excluded WASP, the gene responsible for Wiskott-Aldrich Syndrome and its allelic variant X-linked thrombocytopenia, as the causative gene. GATA-1, a transcription factor required for proper erythroid and megakaryocytic cell development, is contained in the 7.65 KcM minimal linkage region. GATA-1 contains two highly conserved zinc finger domains important in protein-protein and protein-DNA binding. The N-finger is bifunctional, mediating interaction with a cofactor, FOG, and modulating DNA-binding by the C-finger. A missense mutation affecting a residue required for FOG interaction has been observed previously in patients exhibiting anemia plus thrombocytopenia with giant platelets and platelet dysfunction. By sequence analysis, a missense mutation in a different residue of the N- zinc finger was identified in all affected males and carrier females in the XLTT family, but in none of 100 control X chromosomes studied. The GATA-1^{XLTT} mutant is able to rescue substantially a GATA-1-null erythroid cell line for erythroid cell maturation. In vitro studies show that GATA-1 binds normally to FOG, but has an increased off-rate for DNA-binding equivalent to that of a C-finger only GATA factor. This is the first report of a human GATA-1 mutation that reveals the DNA-binding contributions of the N-finger of a GATA-factor. The phenotype of XLTT subjects is evidence that the N-finger is involved in regulating globin synthesis, as well as in platelet production and function. There are multiple GATA-1 binding sites throughout the b globin cluster and in vitro studies to characterize the effect of GATA-1^{XLTT} on globin chain synthesis are underway.

Program Nr: 16 from the 2000 ASHG Annual Meeting

A novel mutation mechanism, insertion of b**-satellite repeats, in a transmembrane protease gene causes the autosomal recessive deafness DFNB10.** *H.S. Scott[1], M. Wattenhofer[1], K. Shibuya[2], A. Berry[3], J. Kudoh[2], M. Guipponi[1], K. Kawasaki[2], S. Asakawa[2], S. Minoshima[2], M.P. Papasavvas[1], C. Rossier[1], R. Chrast[1], M. Korostishevsky[3], N. Shimizu[2], B. Bonne-Tamir[3], S.E. Antonarakis[1].* 1) Div of Med Genetics, Uni of Geneva Med School, Geneva, Switzerland; 2) Dept of Mol Biol, Keio Uni School of Med, Tokyo, Japan; 3) Dept of Human Genetics, Sackler School of Med, Tel Aviv Uni, Israel.

Approximately 50% of early-onset deafness cases are genetic and ~50% of these are recessively inherited. Of twenty-six recessive non-syndromic deafness loci, 6 have been cloned. The critical region for the autosomal recessive nonsyndromic deafness locus, DFNB10, on 21q22.3 was refined to less than 1Mb in a large, consanguineous Palestinian family using 50 precisely mapped microsatellite markers (34 new). Six published genes and 7 new genes were screened for mutations. Amplification and sequence analysis of exon 11 of a novel gene called TMPRSS3 (transmembrane serine protease 3) from a DFNB10 sample revealed an insertion of 18 b-satellite repeats within the protease domain. b-satellites are ~68 bp monomeric repeat units tandemly repeated in arrays of up to several hundred kb present mainly on the short arms of acrocentric chromosomes. The inserted repeats may be derived from recombination of extrachromosomal DNA elements containing b-satellite repeats (spcDNA) with a region of minimal homology in the TMPRSS3 gene. TMPRSS3 has 13 exons over ~30 kb and is expressed in several tissues including fetal cochlea. In addition to the serine protease domain and the transmembrane domain, the TMPRSS3 protein has a LDLRA (low density lipoprotein receptor A) domain and a SRCR (scavenger receptor cysteine-rich) domain. The contents of the endolymphatic sac shows a prominence of glycosylated acidic proteins and turnover of these proteins may require specific proteases. This is the first description of b-satellite insertion into an active gene resulting in a pathogenic state as well as the first description of a protease involved in hearing loss. The elucidation of the function of proteases in hearing loss, and more particularly TMPRSS3, may lead to new therapeutic protocols.

Program Nr: 17 from the 2000 ASHG Annual Meeting

Identification of a novel locus for hearing impairment on chromosome 22q11, a region associated with congenital anomaly disorders. *B. Funke[1], R.K. Pandita[1], D. Madnani[1], C. Shoemaker[1], T. Schüler[2], M.C. Brown[3], T. Van De Water[1], H. Schorle[2], J. Adams[3], B. Morrow[1]*. 1) Albert Einstein College of Medicine, Bronx, NY; 2) Forschungszentrum Karlsruhe, ITG, Eggenstein-Leopoldshafen, Germany; 3) Massachussetts Eye and Ear Infirmary, Boston, MA.

Three congenital disorders, cat-eye syndrome (CES), der(22) syndrome and velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS), result from tetrasomy, trisomy and monosomy, respectively, of part of 22q11. They share a 1.5 Mb region of overlap, which contains more than 25 genes and is conserved on mouse chromosome 16. Novel approaches to determine the molecular basis of the disorders include the generation of large nested deletions and duplications in the mouse. We used human BACs covering most of the distal half of the 1.5 Mb region to generate a panel of transgenic mice. Together, they contain ten known genes, including *TBX1*, *UFD1L*, and *COMT*. Quantitative Southern blot hybridization studies revealed that the BAC copy number varied in different lines. Generally, transgene expression was copy number dependent as determined by Northern blot analysis. Initial analysis revealed that all BAC transgenic mice were viable and fertile. One BAC caused hyperactive circling in two independent lines. Transgenic mice had severe inner and middle ear defects, including hypoplastic semicircular canals, shortened vestibular aqueduct, a reduced number of cochlear turns and a dysmorphic stapes. In addition, mice had mild to severe hearing loss. The observed malformations fit the pattern of defects found in Mondini dysplasia, reported in isolation or in association with other human aneuploidy syndromes, including VCFS/DGS. The mouse ortholog of one of the transgenes (*Tbx1*) is expressed in the precursors of the structures affected in transgenic animals. We showed that the orthologs of the two remaining genes, *Pnutl1* and *Gp1B*b are not expressed in those structures. Using BAC mediated transgenesis, we generated mice with severe inner and middle ear malformations leading to balance problems and hearing loss. We provide evidence that those defects may be caused by overexpression of *TBX1*.

Program Nr: 18 from the 2000 ASHG Annual Meeting

Mapping *DFNB29* and Cloning this Novel Nonsyndromic Deafness Gene on Chromosome 21q22. *E.R. Wilcox[1], S. Naz[4], S. Riazuddin[1,4], T.N. Smith[1], I. Belyantseva[2], Q. Burton[3], T. Ben-Yosef[1], A.J. Griffith[1], R.J. Morell[1], D.K. Wu[3], B. Kachar[2], S. Riazuddin[4], T.B. Friedman[1]*. 1) Laboratory of Molecular Genetics, NIDCD, NIH, Rockville, MD; 2) Section on Structural Cell Biology, LCB, NIDCD, NIH, Bethesda, MD; 3) Section on Sensory Cell Regeneration and Development, LMB, NIDCD, NIH, Rockville, MD; 4) Center of Excellence in Molecular Biology, Lahore, Pakistan.

A large consanguineous family with autosomal recessive profound nonsyndromic deafness defines the locus *DFNB29* on chromosome 21q22. A fully informative linked marker supports a LOD score of 6.3 in this family. The locus maps between markers D21S263 and D21S267. This interval does not overlap the *DFNB8*/*DFNB10* intervals, also on chromosome 21. A second family segregating autosomal recessive profound nonsyndromic hearing loss was ascertained which supported a LOD score of 4.1 for a marker in the *DFNB29* interval. The second family is also from Pakistan and further refined the locus interval. The two families are unrelated and carry different haplotypes for the markers tested in the linkage region. In both families deafness was the one consistent phenotypic trait found among the 13 affecteds with mutations in this gene. A frame-shift mutation in the coding sequence was found upon sequencing a candidate gene in one of the two families. The other family has a missense mutation in a highly conserved amino acid, when comparing the *DFNB29* gene product to related proteins. Neither mutation was found by DNA sequence analysis among more than 150 normal control individuals from Pakistan. The *DFNB29* gene is expressed in the inner and outer hair cells and supporting cells of the organ of Corti. More than 400 affected individuals with inherited deafness are currently being screened for mutations in this gene. Transgenic knock-out and knock-in mutations of the *DFNB29* gene are currently being constructed.

Program Nr: 19 from the 2000 ASHG Annual Meeting

Targeted disruption of mouse *Pds* provides key insight about the inner-ear defects encountered in Pendred syndrome. *L.A. Everett[1], I.A. Belyantseva[2], K. Noben-Trauth[2], R. Cantos[2], A. Chen[1], S.I. Thakkar[1], S.L. Hoogstraten-Miller[1], B. Kachar[2], D.K. Wu[2], E.D. Green[1]*. 1) National Human Genome Research Institute, NIH, Bethesda, MD 20892, USA; 2) National Institute on Deafness and Other Communication Disorders, NIH, Bethesda, MD 20892, USA.

Pendred syndrome (PS) is an autosomal recessive disorder characterized by prelingual sensorineural hearing loss and goitre. Following our identification of the gene defective in PS *(PDS)*, numerous studies have focused on defining the role of *PDS* in deafness and PS as well as elucidating the function of the *PDS*-encoded protein (pendrin); such efforts yielded information about *PDS* expression in the inner ear and thyroid as well as the iodide/chloride anion-transporting specificity of pendrin. Furthermore, *PDS* mutations have now been documented in deaf individuals without thyroid disease, indicating that the gene accounts for a broader spectrum of deafness than just PS. More detailed examination of pendrin function, however, requires a model experimental system. Towards that end, we generated a *Pds*-knockout mouse. $Pds^{-/-}$ mice are completely deaf, but interestingly a subset also display signs of vestibular dysfunction. The inner ears of $Pds^{-/-}$ mice appear to develop normally until embryonic day 15, after which point widespread endolymphatic dilatation occurs, especially of the endolymphatic duct and sac, reminiscent of the radiological findings in deaf individuals with *PDS* mutations. Additionally, on scanning electron microscopy, severe degeneration of the hair cells of the cochlea and vestibule is seen in $Pds^{-/-}$ mice; the otoconia are abnormally sparse and occasional giant otoconia are seen as well as a disrupted otoconial membrane. All of these findings point towards a perturbation of the normal electrolytic equilibrium of the endolymph, presumably due to the lack of pendrin's anion-exchange function. The ultrastructural defects seen in $Pds^{-/-}$ mice thus provide important clues about the mechanisms responsible for the inner-ear pathology associated with *PDS* mutations.

Program Nr: 20 from the 2000 ASHG Annual Meeting

Williams syndrome: Genes and pathways responsible for human cognition. *J.R. Korenberg[1], X-N. Chen[1], Z-Y. Shi[1], E. Schmidt[2], Z. Lai[3], U. Bellugi[3], A. Reiss[2], D. Mills[4], A. Galaburda[5]*. 1) Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, UCLA, CA; 2) Dept. of Child and Adolescent Psychiatry, Stanford Univ, Palo Alto, CA; 3) Laboratory for Cognitive Neuroscience, The Salk Institute, La Jolla, CA; 4) Center for Research in Language, UCSD, La Jolla, CA; 5) Beth Istrael Deaconness Medical Center, Boston, MA.

Wms is a compelling model of human cognition. Due to a deletion of human chromosome band 7q11.2, subjects exhibit striking peaks and valleys in neurocognition (deficits in visual-spatial and global processing, relatively preserved language and face processing, hypersociability, and heightened affect), characteristic neuroanatomical defects (abnormalities of the parietal-occipital junction, the superior temporal gyrus, and cerebellum), and most recently, characteristic features of neuronal pathways as measured by functional MRI (magnetic resonance imaging) and ERPs (event related potentials). To identify the genes whose deletion is responsible for these features, we have generated an integrated physical, genetic and transcriptional map of the WMS and flanking regions and used this to determine the genes deleted and their relationship to phenotype in WMS subjects with the common deletion and in subjects with smaller deletions. We now report the molecular, neurocognitive, neuroanatomic, ERP and functional imaging results on a subject with a smaller deletion that results in a single copy of all genes except for two genes. We have compared the results with this subject to the normed results obtained from diploid controls and WMS with common deletions. The results establish regions and consequent gene candidates for characteristic WMS features. We propose that there are two genes that are responsible in part for the visual-spatial deficits, and global processing, for neuroanatomic defects of the parietal/occipital region, and for distinct neural pathways as reflected in functional MRI and ERPs. These results provide the basis for defining the pathways linking genes with the neuroanatomical, functional and behavioural consequences that result in human cognition.

Program Nr: 21 from the 2000 ASHG Annual Meeting

Clinical importance of routine screening for cryptic translocations in moderate/severe familial MR and MCA/MR patients. *A. Verloes, S. Lesenfants, M. Jamar, V. Dideberg, C. Herens.* Wallonia Ctr Human Genetics, Sart Tilman Univ Hosp, Liège, Belgium.

The importance of cryptic cytogenetic anomalies in MR was suggested by Wilkie (Am J Hum Genet 53:688-701) and confirmed by Flint et al. (Nat Genet 9:132-140). In a wide scale study by the same group (Knight et al. Lancet 354:1676-1681), almost 8% of a group of children with severe mental retardation were shown to have cryptic anomalies involving subtelomeric regions. The authors estimated the incidence of cryptic imbalance to be 1/5000, i.e.twice the incidence of visible anomalies (trisomy 21 excluded.) Initially using sequential subtelomere probing, and, since 1998, the Cytocell Chromoprobe Multiprobe kit, we systematically investigated children with moderate to severe MR fulfilling one of the following criteria 1) at least some minor dysmorphic signs, irrespective of the familial history, 2) familial history of MR or MCA, irrespective of the phenotype or 3) MCA syndrome with facial dysmorphology in the newborn (MR not assessable). At the time of submission, 10 cryptic rearrangements have been found among 114 unrelated patients (> 19 unbalanced subjects), including 2 families previously reported as recessive MCA/MR syndromes (GOMBO ans Lambotte syndromes). In 6/10 patients, a balanced rearrangement was present in one parent. Clinical and cytogenetic details will be presented. All the literature on private, or very uncommon sporadic or recessively inherited MR and MCA/MR syndromes is now suspect to deal with undetected cytogenetic anomalies (cf. Pitt-Roger-Danks, Haspeslagh, pseudo-Albright) Results of telomere screening and perhaps CGH will become a sine qua non condition for publishing such syndromes in the next future. Re-testing previously reported families with those methods will be a priority in dysmorphology during the next few years, both to correct nosology of MCA/MR syndromes and for acurate genetic counseling of the families, as we dramatically showed it with Lambotte syndrome (Herens et al. Am J Med Genet 73:127-131).

Program Nr: 22 from the 2000 ASHG Annual Meeting

Tetralogy of Fallot with characteristic facies: a novel syndrome caused by mutation in *Jagged1*. *Z. Eldadah[1], A. Hamosh[1], F. Vermeiren[2], H.C. Dietz[1]*. 1) HHMI and Inst of Genetic Med, Johns Hopkins Univ; 2) Acuson Corp, Baltimore, MD.

Tetralogy of Fallot (ToF) is the most common form of complex congenital heart disease (1 in 3,000 live births). Evaluation of candidate loci in a large kindred with autosomal dominant ToF with reduced penetrance culminated in the identification of a missense mutation (G274D) in *Jagged1*(JAG1), a Notch ligand expressed in the developing right heart and involved in cell fate determination. Fourteen members of the kindred carried this mutation, including 1 deceased and 2 living individuals with ToF, and all obligate carriers. No DNA was available for 2 deceased sibs with ToF born to a G274D carrier. Of the 11 available for clinical and echocardiographic examination, all but one had cardiac disease, including 2 with classic ToF, 2 with ventricular septal defect, and 6 with peripheral pulmonic stenosis (PPS). All forms of ToF were represented in this kindred, including pulmonary stenosis, pulmonary atresia, and absent pulmonary valve.

Haploinsufficiency of JAG1 causes Alagille syndrome (AGS), a rare, dominant disorder with biliary atresia, growth retardation, vertebral and ocular anomalies, cognitive deficits, characteristic facies, and right-sided heart defects, predominantly PPS. In sharp contrast to patients with AGS, no individual in this family showed clinical, chemical, or histological evidence of cholestasis. Moreover, growth and cognition were normal, and no specific abnormalities of the eye or vertebral column were documented. All G274D carriers had a characteristic face with long, upslanting palpebral fissures, prominent and high zygomatic arches, broad nasal bridge, flat anterior maxilla, and broad chin. This was easily distinguishable from the AGS facies. Glycine is highly conserved at the position corresponding to residue 274 in EGF-like domains of *Jagged1* and other proteins. Mutation of this glycine in factor IX and fibrillin-1 has been associated with atypical variants of disease. Taken together, these data suggest that a gain-of-function pathogenetic mechanism underlies the phenotype in this family and that JAG1 mutations may contribute significantly to the broad spectrum of right heart obstructive disease.

Program Nr: 23 from the 2000 ASHG Annual Meeting

Genotypic and phenotypic spectrum in the tricho-rhino-phalangeal syndromes types I and III. *G.G. Gillessen-Kaesbach[1], J. Schaper[2], P. Momeni[1], B. Horsthemke[1], H.-J. Lüdecke[1]*. 1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany; 2) Zentralinstitut für Röntgendiagnostik, Kinderradiologie, Universitätsklinikum, Essen, Germany.

The tricho-rhino-phalangeal syndrome (TRPS) is characterized by craniofacial and skeletal abnormalities. Three subtypes have been described. TRPS I is caused by mutations in the *TRPS1* gene on chromosome 8. TRPS II is a microdeletion syndrome affecting the *TRPS1* and *EXT1* genes. One form of TRPS with severe brachydactyly and growth retardation has been called TRPS III. To establish a genotype-phenotype correlation, we searched for *TRPS1* gene mutations in 51 unrelated patients with TRPS I or III. Twenty patients have a positive family history for TRPS. We found 35 different mutations in 44 cases (detection rate 86 %). This indicates that *TRPS1* is the major if not the only locus for TRPS types I and III. We did not find any mutation in the parents of sporadic cases or apparently healthy relatives of familial cases, indicating complete penetrance of *TRPS1* mutations. We found nine out-of-frame deletions (1-14 bp) and seven out-of-frame insertions (1-4 bp) in TRPS1. Of the 19 single-base substitutions, nine are transversions and ten are transitions. Six transitions occur in CpG dinucleotides, and four of them are recurrent mutations. All frame-shift mutations and thirteen base substitutions create premature stop codons. One base substitution leads to abnormal splicing of the *TRPS1* transcript. All five missense mutations alter the GATA DNA-binding zinc-finger.We found them in patients with TRPS III. The examination and metacarpophalangeal pattern profile (MCPPP) analysis of the hand radiographs of 43 patients revealed that a TRPS-specific pattern does not exist. There is even no correlation of the MCPPP's of unrelated patients with either TRPS I or III and identical mutations. Nevertheless, the MCPPP's of patients with TRPS I and III differ significantly. We conclude that TRPS III is not a separate entity, but only one extreme of the phenotypic spectrum caused by a specific class of mutations in the *TRPS1* gene.

Program Nr: 24 from the 2000 ASHG Annual Meeting

Heterozygous mutations in the gene ZIC2 are a significant cause of sporadic holoprosencephaly (HPE): 15 cases; recurrence of an alanine tract expansion. *S.A. Brown[1], L.Y. Brown[1], M. Muenke[2]*. 1) Obstetrics and Gynecology, Columbia University, New York, NY; 2) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD.

ZIC2 is a vertebrate homologue of the Drosophila gene, odd-paired or opa. We have previously reported that ZIC2 is located in the 13q32 critical deletion region and that heterozygous mutations result in HPE. Others have recently shown that a diminished level of Zic2 protein in mice results in a brain malformation which is similar to human HPE. We now report the results of mutation screening of approximately 400 HPE patients. Overall, 15 (~3.5%) of HPE samples were shown to have ZIC2 mutations. All of the cases had either alobar or semi-lobar HPE. Although about 85% of HPE patients in general have severe facial malformation, it is striking that none of those with ZIC2 mutation had significant facial malformation. Thus ZIC2 mutation cases appear to represent a subset of HPE in which the face does not predict the brain. Of the mutations we encountered, 8 were frameshifts which are predicted to result in loss of function and functional hemizygosity. Six of the cases were caused by an alanine tract expansion which, although it is a triplet repeat, most likely represents an unequal cross over event rather than microsatellite instability. In three of these 6 cases, one of the parents of the affected individual is apparently a mosaic carrier of the mutation. This finding suggests that, in these cases, the mutation occurred during a somatic recombination event. Overall, our results firmly establish ZIC2 mutation as a cause of human brain malformation and help to define the range of phenotypes associated with ZIC2 malformation.

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Program Nr: 25 from the 2000 ASHG Annual Meeting

GLI3 deletions associated with Greig cephalopolysyndactyly and implications for phenotypic overlap with Acrocallosal syndrome. *I.M. Olivos-Glander[1], J. Blancato[2], J. Meck[3], L.G. Biesecker[1].* 1) GDRB, NHGRI, NIH, Bethesda, MD; 2) Inst. of Mol. Hum. Gen., Georgetown Univ., Washington, D.C; 3) Dept of OB/GYN, Georgetown Univ., Washington, D.C.

Greig cephalopolysyndactyly syndrome (GCPS) is an autosomally inherited disorder caused by the haploinsufficiency of GLI3. The phenotype includes preaxial or postaxial polydactyly of the limbs and macrocephaly and/or hypertelorism. Additional characteristics may include mental retardation, hernias and dysgenesis of the corpus callosum. This phenotype overlaps extensively with acrocallosal syndrome (ACLS). A wide range of genetic alterations in GLI3 is reponsible for GCPS and we are using a systematic diagnostic protocol to assess them. This protocol can also serve to accurately diagnose individuals with this phenotype and distinguish GCPS from ACLS.

The protocol initially assays for macro deletions with high resolution karyotyping. We use both microsatellite and FISH analysis to assess submicroscopic deletions and finally, biallelic cDNA expression assays and sequencing is performed. At this time, we have identified that 10 of the 29 affected probands have large genomic deletions and three of six patients were found to be monoallelic for GLI3 by the biallelic expression assay. In total 14 of the 29 families evaluated so far are found to have a genetic aberration in GLI3. Six patients found to be deleted for GLI3 by FISH analysis are also deleted for the proximal telomeric gene, INHBA. All six of these patients belong to a subset of GCPS patients who are developmentally delayed with some dysgenesis of the corpus callosum. Therefore, this extensive deletion suggests that these patients should be diagnosed with a contiguous gene deletion syndrome.

This diagnostic protocol can not only determine GLI3 alterations in GCPS patients, but it may also determine if some patients with overlapping phenotypes, such as ACLS, also have genetic aberrations around GLI3. Our data suggests that a contiguous gene deletion which includes GLI3, can produce a phenotype indistinguishable from ACLS.

Program Nr: 26 from the 2000 ASHG Annual Meeting

Relationship of Madelung's deformity to Leri-Weill syndrome and SHOX deletions. *A.R. Zinn[1], A.L. Osterman[2], C.I. Scott Jr.[3], L. Nicholson[3], J.L. Ross[4].* 1) McDermott Center, University of Texas Southwestern Medical School, Dallas, TX; 2) Department of Orthopedic Surgery, Thomas Jefferson University, Philadelphia, PA; 3) Alfred I. duPont Hospital for Children, Wilmington, DE; 4) Department of Pediatrics, Thomas Jefferson University, Philadelphia, PA.

BACKGROUND: Madelung's wrist deformity is a radiologic diagnosis that includes forearm shortening with radial bowing, premature fusion of the ulnar half of the radial epiphysis, dorsal dislocation of the distal ulna, and wedged carpal bones between the deformed radius and the protruding ulna. Previously, most Madelung's patients were thought to have Leri-Weill dyschondrosteosis (LWD; Madelung's, mesomelia, and short stature). LWD can now be defined genetically by deletion or mutation of the SHOX gene in 60% of cases.

AIMS: We have studied a group of 17 families with member(s) having bilateral Madelung's deformity (23 affecteds, ages 5-66 yr) in order to 1) investigate the relationship between SHOX deletions, Madelung's deformity, and other skeletal features (height, limb length, metacarpal length, carrying angle, and palate morphology), and 2) examine the overlap between Madelung's and LWD.

RESULTS: SHOX deletions were detected by FISH in affected individuals from 5/7 LWD versus 0/10 normal stature (height SDS > 1.5 SD) families. Distal Xp deletions were found by karyotype in 3 LWD families. Mutational analysis of SHOX in non-deleted patients is in progress. No clinical features clearly distinguished LWD subjects with or without SHOX deletions. Mesomelia was the only associated feature that distinguished LWD versus normal stature Madelung's subjects.

CONCLUSIONS: SHOX deletions were present in 71% of LWD families. Physical features such as increased carrying angle, high-arched palate, or shortened metacarpals were not specific for LWD or SHOX deletions. Contrary to prior assumptions, Madelung's deformity also occurs in normal stature individuals without mesomelia and is not associated with SHOX deletions in these patients. Lastly, chromosome abnormalities are a relatively frequent cause of LWD.

Program Nr: 27 from the 2000 ASHG Annual Meeting

CBP mutation detection and genotype-phenotype correlation in patients with Rubinstein-Taybi syndrome (RTS) and a woman with presumably a mild RTS "variant" and normal intelligence. *O. Bartsch[1], K. Locher[2], P. Meinecke[3], W. Kress[4], M. Pilz[1], K. Ostermann[2], G. Rödel[2]*. 1) Institut für Klinische Genetik, Technische Universität Dresden, Germany; 2) Institut für Genetik, TU Dresden, Germany; 3) Abteilung Medizinische Genetik, Altonaer Kinderkrankenhaus, Hamburg, Germany; 4) Institut für Humangenetik, Universität Würzburg, Germany.

We studied 45 unrelated patients with Rubinstein-Taybi syndrome (RTS) using FISH and disclosed four patients with a deletion of the CBP gene. We then screened a subset of 16 patients from this cohort and a case of a presumably mild RTS "variant" for molecular mutations (CBP-cDNA, 7329 bp, 2442aa). Methods included PCR fragment length analysis with cloning and sequencing of abnormal fragments, single strand conformation analysis (SSCP) and direct genomic sequencing (DGS) of individual exons and splice sites. A total of 5 sequence variants, including 4 genuine pathogenic mutations, were identified. All are novel when compared to previous reports (Petrij et al Nature 1995;376:348, Giles et al AJHG 1999;61S:A297). Three RTS patients were found to have mutations clustering in the 5'-terminal region of the CBP gene (exon2, 86del148-bp; exon4, C1108T; intron7, 1676+1G®T), resulting in a frameshift with premature truncation codon or a stop codon (aa28, 37PTC; R370X; aa559, 560PTC) and a grossly truncated protein without functional CREB-binding domain (which spans from aa452 to aa684). The 18-yr-old female with a clinical diagnosis of possibly mild RTS was found to have a missense mutation (exon18, A3524G) predicting a non-conservative aa change (Y1175C) in the CBP bromodomain (SWISS-PROT database) or histone acetyltransferase domain (Giles et al Genomics 1997;42:96), a region where no mutation has been reported to date. She had an only mild expression of RTS with subtle craniofacial features, broad thumbs and broad big toes, but she had normal height and head circumference and - even more remarkably - low but normal intelligence. Results are pending in the other patients. In summary, we report here findings supporting that RTS results from human CBP null alleles and, interestingly, the first patient with atypical RTS and an unusual CBP mutation.

Program Nr: 28 from the 2000 ASHG Annual Meeting

Relative undermethylation of the SNRPN locus in a girl with features of Angelman and Prader-Willi syndromes.

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Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are mental retardation syndromes caused by the lack of expression of maternal and paternal genes, respectively, from the imprinted chromosomal region 15q11-13. AS is characterized by a happy disposition with paroxysms of laughter, flexed arms, absent speech, ataxia, seizures, and sleep disturbance. Features of PWS include neonatal hypotonia, failure to thrive in infancy followed by hyperphagia and obesity in early childhood, small hands and feet, and hypogenitalism. Recently, several children have been reported with overlapping features of both syndromes and a unique Southern blot pattern consisting of a faint but not absent maternal SNRPN band reminiscent of AS. We report our clinical and laboratory investigations of another such child. A 4 yo girl presented to our Genetics Clinic with clinical features of AS, obesity, and hypotonia. She was born to a 37 yo mother by intracytoplasmic sperm injection because of paternal testicular cancer. Cytogenetic studies on blood and fibroblast revealed a normal 46, XX female karyotype and two copies of the SNRPN locus by FISH. Microsatellite analysis confirmed biparental inheritance of chromosome 15. DNA methylation studies of SNRPN by Southern blotting showed a faint maternal band and a strong paternal band indicating relative undermethylation. DNA sequencing of the 880 bp AS imprinting center-SRO identified no mutations. Possible mechanisms for this distinctive AS-PWS phenotype include somatic mosaicism for one of the molecular mechanisms responsible for AS, or alternatively, a novel germ-line mutation that causes incomplete methylation of one or more maternal genes within chromosome 15q11-13.

Program Nr: 29 from the 2000 ASHG Annual Meeting

Two affected males in a Rett syndrome family : clinical and molecular findings. *L. Villard[1], A. Kpebe[1], C. Cardoso[1], J. Chelly[2], M. Tardieu[3], M. Fontès[1].* 1) Faculté de Médecine, INSERM U491, Marseille, France; 2) CHU Cochin, INSERM U129, Paris, France; 3) Sce de Neurologie Pédiatrique, Hopital du Kremlin Bicêtre, Paris, France.

We report a familial case in which two boys were affected with severe neonatal encephalopathy of unknown aetiology. They both deceased by one year of age of severe apnea. They have a sister affected with a classical form of Rett syndrome. Since mutations in the methyl-CpG-binding protein 2 (MECP2) gene were identified in 70 to 80% of Rett syndrome sporadic cases, we looked for a mutation in the MECP2 gene in this family. We have identified a missense mutation in the affected girl and subsquently showed that one of her affected brother, for which DNA was available, also carried the same mutation. The mother of the patients is a carrier of the mutation and we used X-chromosome inactivation studies to show that she has a completely skewed X-chromosome inactivation pattern which favors the expression of the normal MECP2 allele and thus probably explains why she does not exhibit any phenotypic manifestation. In addition, we have haplotyped the whole X chromosome using 30 polymorphic markers distributed evenly on the human X chromosome and we show that the MECP2 mutation appeared on the grand-paternal X chromosome. These findings represent the second reported case of a male affected with a MECP2 mutation can be identified in boys, that they do not present a Rett syndrome phenotype and ask the question about the frequency with which this event occurs.

Program Nr: 30 from the 2000 ASHG Annual Meeting

Abnormal brain structure and function in adult males with non-syndromic clefts of the lip and/or palate. *P. Nopoulos, S. Berg, L. Richman, J. Canady.* University of Iowa Hospitals and Clinics, Iowa City, Iowa.

Objective: The aim of this study was to determine if adult males with non-syndromic clefts of the lip and/or palate (CLP) have abnormal brain morphology and/or cognitive dysfunction. Brain morphology (using MRI) and performance on cognitive tasks in a sample of 50 males with non-syndromic CLP were analyzed and compared to 50 healthy controls matched for sex, age, and parental socioeconomic status.

Results: The males with CLP have significantly lower full scale, verbal, and performance Intelligence Quotients (IQ). After controlling for full scale IQ, subjects had significant deficits in several language tasks, but no deficit in visuospatial skill.

In regard to brain morphology, CLP subjects had a robust anterior to posterior tissue distribution shift. That is, CLP patients had significantly *larger than normal* frontal and parietal lobes, but significantly *smaller than normal* temporal lobes, occitipal lobes and cerebellar volume. The abnormality in the temporal lobes were most severe on the left side, an area of the brain specialized for language function.

In addition, subjects with CLP had an elevated incidence of a specific neurodevelopmental brain anomaly, enlarged Cavum Septi Pellucidi (CSP). Moreover, Full Scale IQ was significantly and inversely correlated with this anomaly indicating the larger the anomaly, the lower the IQ.

Conclusions: Adult males with CLP have abnormal brain structure and function. Against the background of mild global cognitive dysfunction, language function is particularly disturbed. In addition, adult males with CLP have a significantly altered pattern of brain morphology compared to healthy controls. This abnormal brain structure is most likely due to aberrant cerebral development,and is directly related to the cognitive dysfunction. This study highlights the complex interaction and interdependence of craniofacial and cerebral development.

Program Nr: 31 from the 2000 ASHG Annual Meeting

The location and type of mutation predict malformation severity in isolated lissencephaly caused by abnormalities within the *LIS1*gene. *C. Cardoso[1], R.J. Leventer[1,2], N. Matsumoto[3], J.A. Kuc[1], S.K. Mewborn[1], L.L. Dudlicek[1], M.B. Ramocki[1], S. Das[1], P.L. Mills[1], D.T. Pilz[4], W.B. Dobyns[1,2], D.H. Ledbetter[1].* 1) Departments of Human Genetics and; 2) Neurology, University of Chicago, Chicago, IL; 3) Human Genetics, Nagasaki University School of Medicine, Japan; 4) Institute of Medical Genetics, University Hospital of Wales, United Kingdom.

Lissencephaly is a cortical malformation secondary to impaired neuronal migration resulting in mental retardation, epilepsy and motor impairment. It may occur in isolation or as part of the Miller Dieker syndrome. There is a severity spectrum from agyria with a severely thickened cortex to posterior band heterotopia only (grades 1-6). The LIS1 gene on 17p13.3 encodes a 45 kDa protein (PAFAH1B1) containing 7 WD repeats and required for correct neuronal migration. Approximately 65% of patients with isolated lissencephaly sequence (ILS) show mutations or deletions of the LIS1 gene. We have identified 30 ILS patients with mutations of LIS1 and no deletion by FISH. We found 5 splicing defects and 5 missense, 5 nonsense and 15 frameshift mutations. 16 of the mutations are novel. We analyzed the correlation between genotype and malformation severity grade in 26 patients with available neuroimaging. Severity ranged from grade 2 to grade 5. Missense mutations were found in exons 3, 6 and 9. Patients with missense mutations had a milder lissencephaly grade compared to those with protein truncation mutations ($p=0.013$). Protein truncation mutations were found throughout the gene except in the third WD repeat coded by exon 7. These patients were divided into two groups depending on whether the mutation occurred before or after the first WD repeat (early vs. late protein truncation respectively). Early protein truncation produced a more severe lissencephaly than late truncation ($p=0.003$). Our results suggest that the severity of lissencephaly in ILS caused by LIS1 mutations may be predicted by the type and location of the mutation. We confirm the importance of the microtubule-binding and WD repeat domains for LIS1 protein function and suggest that the small number of missense mutations identified may be due to underdiagnosis of less severe phenotypes.

Program Nr: 32 from the 2000 ASHG Annual Meeting

Melanocortin-1 receptor genotype is a risk factor for basal cell carcinoma, squamous cell carcinoma and melanoma. *R.A Sturm[1], N.F. Box[1], D.L. Duffy[2], J.S. Palmer[1,2], W. Chen[1], J.F. Aitken[2], P.G. Parsons[2], A.C. Green[2], N.K. Hayward[2], N.G. Martin[2]*. 1) CMCB, Institute for Molecular Bioscience, University of Queensland; 2) QIMR, Brisbane, Queensland, Australia.

The MC1R gene encoding the melanocyte-stimulating hormone receptor is highly polymorphic in the Caucasian population, with three variant alleles (R151C, R160W and D294H) shown to be associated with red hair, fair skin color and skin UV-sensitivity. We have analysed the frequency of these alleles in an Australian population-based sample of 111 high- and 109 low-risk basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) individuals. An association between BCC, SCC, and solar keratosis was demonstrated for each of these active alleles (OR = 3.15; 95%CI 1.7, 5.82), whereas assay of other three common variants (V60L, V92M and R163Q) were found to have minimal association with pigmentation phenotype or BCC, SCC and solar keratosis risk. A significant heterozygote effect was demonstrated with individuals carrying a single MC1R variant allele more likely to have fair and sun-sensitive skin as well as solar lesions when compared to those individuals with two consensus MC1R alleles. Melanoma is also a common cancer in the Australian Caucasian population and we have identified a strong association of the former three MC1R variants with melanoma using 459 melanoma cases and 399 matched control individuals ascertained from the southeast Queensland population. This larger study confirmed the importance of MC1R in determining red hair color, and fair skin. Moreover, the risk of melanoma found to increase two-fold for individuals carrying one variant allele (OR = 2.2; 95%CI 1.6-3.0) and four-fold if both alleles were variant (OR = 4.1; 95%CI 2.1, 7.9). Among pale-skinned individuals, this association between melanoma and MC1R variants was absent when stratified on the basis of hair color, but persisted among those reporting a medium or olive/dark complexion. We conclude the effect MC1R variant alleles have on melanoma is partly mediated via determination of pigmentation phenotype and that some alleles may negate the protection normally afforded by darker skin coloring.

Program Nr: 33 from the 2000 ASHG Annual Meeting

Geographical variation in CDKN2A penetrance for melanoma. *D.T. Bishop[1], A. Goldstein[2], F. Demenais[3], Melanoma Genetics Consortium[1]*. 1) Gen Epid Lab,St James Univ Hos, Imperial Cancer Research Fund, Leeds, England; 2) NCI Genetic Epidemiology Branch, Bethesda, USA; 3) INSERM, Paris, France.

Germline CDKN2A mutations are the major cause of inherited predisposition to melanoma. The Melanoma Genetics Consortium has as part of its research focus an understanding of the role of CDKN2A in melanoma predisposition. Member groups of the Consortium are based in Australia, Europe and North America and have been identifying mutations in CDKN2A in families ascertained for the occurrence of multiple cases of melanoma. The focus of this study is the estimation of penetrance of CDKN2A germline mutations and the examination of factors which might mediate the penetrance 1) the exposure of family members to non-genetic risk factors, 2) a birth cohort effect paralleling the increase in melanoma incidence world-wide and 3) the co-occurrence of p14ARF mutations in the families. CDKN2A and p14ARF share a common second exon read in different frames; as these two genes are each involved in cell cycle regulation, mutations which impacted both genes might be expected to have an higher penetrance. To date, 80 multiple case families have been identified among eight research groups; these families have in total 402 cases of melanoma recorded (5.0 per family on average). Thirty-seven distinct mutations have been identified in these families; only 9 mutations were observed in more than one family (the most common being the Gly101Trp mutation in 16 families). Penetrance estimation has been performed using two separate approaches; the first involves a conditional likelihood approach and the second logistic regressive modelling. The results using the two approaches are similar indicating overall an estimated risk to age 80 years of approximately 0.70. Statistical evidence for a geographical effect was found, as the estimated penetrance was higher in Australia (0.91 to age 80 years) than in Europe (0.58) with the USA being intermediate in risk (0.76). The significance of the result varied with assumptions made for the statistical analysis. No evidence was found for a birth cohort effect on risk or of increased risk in families carrying both a CDKN2A and a p14ARF mutation.

Program Nr: 34 from the 2000 ASHG Annual Meeting

Toward a gene at Xq27.3 responsible for hereditary prostate cancer (HPC-X). *D.A. Stephan[1,2], G. Howell[5], J. Bailey-Wilson[2], J.R. Smith[2], J. Schleutker[2], A. Baffoe-Bonnie[2], P. Hu[2], S. Joseph[5], L. Malechek[2], N. Papadopolous[4], C.R. Robbins[2], D. Gildea[2], I. Makalowska[2], J.D. Carpten[2], L. Bubendorf[2], M. Hieskanen[2], I. Zucchi[2], W. Isaacs[3], J.M. Trent[2], and the Prostate Investigation Group[2].* 1) Research Center for Genetic Medicine, CNMC, Washington, DC; 2) National Human Genome Research Institute, NIH, Bethesda, MD; 3) Johns Hopkins University, Baltimore, MD; 4) Columbia University, NY, NY; 5) Sanger Centre, Cambridge, UK.

An estimated 5-9% of prostate cancers are inherited as a single-gene Mendelian trait. A genome-wide scan and subsequent high-density focused genotyping has identified a locus at Xq27.3 (termed HPC-X, Xu et. al., Nature Genetics, 1999) which accounts for 40% of hereditary cases in Finland. We have physically mapped 12 Mb in BACs (with 4 gaps) surrounding this locus, including mapping ESTs. Genetic association studies were performed on all of the Finnish families, including sib-pairs, and yielded significant scores with a single marker allele. An Ashkenazi population was ascertained (RB) and genotyped with the identical focused genotyping markers under the linkage peak yielding significant association at a different allele of the identical marker and confirming the linkage disequilibrium. The extent of the haplotype was defined by identifying novel CA repeats and SNPs in the 150kb surrounding the associated markers and the core IBD haplotype was defined in both populations. The BAC encompassing this 150kb was sequenced (as was the entire ~500kb region around the disequilibrium marker) and yielded 3 putative coding regions (using the Genemachine suite of algorithms). The genomic structure of this region is extremely complex, complicating mutation detection. Since it is difficult to predict the type of mutation responsible to an X-linked hereditrary cancer, we are employing a combination of SSCP, northern blot, Southern blot, fiber-FISH and direct sequencing to find the etiological mutation. In addition, we have evidence in our populations if large-scale genomic polymorphisms which may predispose to the phenotype.

Program Nr: 35 from the 2000 ASHG Annual Meeting

Association of HPC2/ELAC2 Genotypes and Prostate Cancer. *T.R. Rebbeck, A.H. Walker, C.M. Zeigler-Johnson, S. Weisburg, K.L. Nathanson, A.J. Wein, S.B. Malkowicz.* Dept of Biostatistics & Epidemiology and Cancer Center, University of Pennsylvania, Philadelphia, PA.

HPC2/ELAC2 is a novel and recently cloned prostate cancer (CaP) susceptibility gene on chromosome 17p that can explain hereditary patterns of CaP in families (Tavtigian et al. 2000). In addition to rare, disease-associated mutations, two common missense variants in HPC2/ELAC2 have been identified: a serine to leucine change at amino acid 217 (Ser217Leu), and an alanine to threonine change at amino acid 541 (Ala541Thr). To evaluate whether these missense variants contribute to CaP etiology, we studied 359 incident CaP cases and 266 age- and race-matched control men. All men were ascertained from a large health system population without regard to family history or other CaP risk factors. Among controls, the Thr541 allele frequency was 2.9% and the Leu217 allele frequency was 31.6%, with no significant differences in frequency across racial groups. Thr541 was only observed in men who also carried Leu217. The probability of having CaP was significantly increased in men who carried the Leu217/Thr541 variants (OR=2.4, 95%CI: 1.1-5.3). This risk did not differ significantly by family history or race. Prostate cancer cases with the Leu217/Thr541 genotype also had a 44% higher mean serum PSA at diagnosis (p=0.018) than men who did not carry this genotype. Using standard attributable risk calculations, genotypes at HPC2/ELAC2 were estimated to explain 5% of CaP in the general population of inference. The present results suggest that common variants at HPC2/ELAC2 are associated with CaP risk and may explain a substantial proportion of CaP in the population.

Program Nr: 36 from the 2000 ASHG Annual Meeting

Association of CYP3A4 variant with prostate cancer in African Americans is due to population stratification. *W. Chen[1], R.A. Kittles[1,2], R.K. Panguluri[1], C.A. Adebamowo[3], C. Ahaghotu[2], F. Ukoli[2], L. Adams-Campbell[2], G. Dunston[1,2], V. Freeman[4]*. 1) National Human Genome Center at Howard University; 2) Howard University Cancer Center; 3) University College Hospital, Ibadan, Nigeria; 4) Loyola University Medical Center, Maywood, IL.

The CYP3A4 gene is a member of the cytochrome P-450 supergene family and is involved in the oxidative deactivation of testosterone. An A to G SNP in the nifedipine specific element of the 5' regulatory region of the gene was recently associated with prostate cancer (Pca) in African Americans. Since the African American population is genetically heterogeneous due to its African ancestry and admixture with Euroamericans, case-control studies using African Americans are susceptible to spurious associations. This is important because allele frequencies for this SNP differ significantly between ethnic groups ($p<0.0001$). In light of this, we tested whether the CYP3A4 SNP was associated with Pca using prostate cancer cases along with age and ethnicity matched controls representing Africans (Nigerian, 73 cases and 82 controls), European Americans (Chicago and DC, 120 cases and 96 controls), and African Americans (DC, 107 cases and 87 controls). Genotyping was performed using PCR and restriction digestion. Observed and expected genotype frequencies were compared between groups and between cases and controls using the two-sided Pearson x^2 test. Results revealed sharp differences in allele frequencies between Nigerian and European American controls (0.86 and 0.11, respectively; $p>0.0001$). African Americans were intermediate at 0.60. No association was observed between CYP3A4 genotype and Pca or related clinical characteristics in Nigerians or European Americans. However, significant association was observed between genotypes (AG and GG dominant model) and Pca in African Americans ($p=0.009$). We subsequently typed 10 unlinked and unrelated SNPs and found significant associations ($p<0.01$) with Pca in African Americans for 3 of the 10 markers. These results reveal the high potential for confounding of association studies using African Americans and the need for study designs that include populations ancestral to African Americans.

Program Nr: 37 from the 2000 ASHG Annual Meeting

Association of a novel E-Cadherin gene coding region SNP with prostate cancer in a large population-based study. *P.A. Koivisto[1], T. Ikonen[1], M. Matikainen[1], N. Mononen[1], H.J. Helin[2], S. Tommola[2], T. Tammela[1], J. Schleutker[1], O-P. Kallioniemi[3]*. 1) Laboratory of Cancer Genetics, IMT, University of Tampere, Tampere, Finland; 2) Department of Pathology, Tampere University Hospital, Tampere, Finland; 3) Cancer Genetics Branch, NHGRI, NIH, USA.

In our recent cancer registry-based study, the incidence of gastric carcinoma was increased up to 5-fold in male relatives of early-onset prostate cancer patients. This association may reflect the influence of genetic factors predisposing to both tumor types. CDH1 gene at 16q codes for the epithelial (E)-cadherin, which plays a key role in the maintenance of intercellular adhesion, cell polarity and tissue architecture. Germline mutations of CDH1 have recently been associated with familial gastric cancer. Since two genome-wide linkage studies of prostate cancer also reported positivity at 16q, we decided to investigate, whether the CDH1 gene mutations would be involved in predisposing to prostate and gastric cancer. No truncating or splice-site CDH1 mutations were identified by PCR-SSCP analysis among 15 Finnish prostate-gastric cancer families and 8 individual patients who had both prostate and gastric cancer. However, a novel S270A missense mutation in exon 6 of the CDH1 gene was seen in one family with 4 prostate and 2 gastric cancers. A large-scale population-based survey of over 1500 DNA samples indicated a significantly (p=0.01-0.02) higher prevalence of S270A among both familial prostate cancer cases (3.3%, n=120) as well as in unselected prostate cancer patients (1.9%, n=592) as compared with blood donors serving as population controls (0.5%, n=923). We conclude that individual rare mutations and polymorphisms in the CDH1 gene, such as the S270A, may contribute to the onset of prostate cancer and warrant further investigations in other populations. CDH1 gene does not, however, appear to explain the link between prostate and gastric cancer.

Program Nr: 38 from the 2000 ASHG Annual Meeting

Impact of gender and parent-of-origin on phenotypic expression of hereditary non-polyposis colon cancer (HNPCC) in 12 Newfoundland families with a founder MSH2 mutation. *J. Green[1], M. O'Driscoll[1], A. Barnes[1], E. Maher[2], K. Shields[3], P. Bridge[3], P. Parfrey[1]*. 1) Disciplines of Medical Genetics and Medicine, Memorial Univ, St.Johns, NF; 2) Section of Med/Molecular Genetics, Univ of Birmingham, UK; 3) Molecular Diagnostic Laboratory, Alberta Children's Hospital, Calgary, AB.

A group of 12 families with a founder MSH2 mutation (nt943+3 A to T) has been identified from a genetic isolate in Newfoundland. Medical records were reviewed to identify types and age at onset of cancer, and age at death. Genetic testing was offered to those at 50% risk. Ascertainment bias was limited by analyzing only sibships where >50% had known genetic status (N=302). 147 were mutation positive (87 clinically affected), 94 mutation negative, and 61 of unknown mutation status. **METHODS:** Kaplan-Meier life table analysis was used to calculate cumulative age to death, and age to onset of any cancer, of CRC, and of endometrial or ovarian cancer. Relative risk was estimated by calculating hazard ratios using a Cox regression model. **RESULTS:** The age-related risks of any cancer, of CRC, or of death from cancer were significantly higher in males than in females (RR=1.7, p=0.02; RR=2.5, p=0.001; RR=2.0, p=0.03 respectively). 24% of women at risk developed endometrial cancer by age 50, and 70% by age 70; whereas 24% developed ovarian cancer by age 50, and 37% by age 70. The mutation was transmitted by the mother (N=91) more frequently than by the father (N=47), associated with the decreased longevity and decreased fertility in affected males. Females who inherited the mutation from their father had a 3.5 RR (p=0.001) of developing any cancer, and a 4.1 RR (p=0.003) of dying from cancer compared to those who inherited the mutation from their mother. **DISCUSSION:** Investigation of large kindreds from the same geographic area and sharing the same mutation may provide new insights into the determinants of phenotype. Search for modifiers of the expression of HNPCC mutations should take account of the difference in risk of cancer, and risk of death from cancer, depending on the sex of the mutation-carrier, and of the parent transmitting the mutation.

Program Nr: 39 from the 2000 ASHG Annual Meeting

Germline hMLH3 mutations in patients with suspected HNPCC. *Y. Wu[1], M.J.W Berends[2], R.G.J Mensink[1], E. Verlind[1], R.H. Sijmons[1], A.G.J. van der Zee[4], H. Hollema[3], J.H. Kleibeuker[2], C.H.C.M. Buys[1], R.M.W. Hofstra[1]*. 1) Dept Medical Genetics, Univ Groningen, Groningen, Netherlands; 2) Dept Gastroenterology, Univ Groningen, Groningen, Netherlands; 3) Dept Pathology, Univ Groningen, Groningen, Netherlands; 4) Dept Gynaecology, Univ Groningen, Groningen, Netherlands.

Hereditary nonpolyposis colorectal cancer (HNPCC), as defined by the so-called Amsterdam criteria, is an autosomal dominant disorder. So far, in five different mismatch repair genes germline mutations have been found associated with HNPCC. These genes account for 50-75% of all HNPCC families. A role of the MutL homologue hMLH3 in HNPCC has not yet been reported. We scanned hMLH3 for mutations in all 11 exons by use of DGGE followed by sequencing of aberrant DNA fragments in patients from 17 unrelated HNPCC families meeting the classic Amsterdam criteria and 208 index patients with suspected HNPCC. We detected seven germline mutations of hMLH3, all in patients suspected for HNPCC. None of the mutations were found in over 200 control individuals. The mutations included one frameshift mutation (2578delA) and six missense mutations (Gln24Glu, Glu624Gln, Arg647Cys, Ser817Gly, Gly981Ser and Ala1370Thr) that all result in substitutions of amino acids belonging to different polarity groups. We could obtain tumor materials for MSI analysis from five of the seven patients with germline hMLH3 mutations. We found that the colorectal carcinoma from the patient with the frameshift mutation displayed an MSI-low phenotype. Tumors from the other patients with the missense mutations in two cases proved MSI-high, in two other cases MSI-low. Our results show involvement of hMLH3 in a proportion of patients with suspected HNPCC.

Program Nr: 40 from the 2000 ASHG Annual Meeting

Altered AXIN2 in Colorectal Cancer with Defective Mismatch Repair. *W. Liu, X. Dong, M. Mai, R.S. Seelan, K. Taniguchi, K.K. Krishnadath, K.C. Halling, J.M. Cunningham, C. Qian, E. Christensen, P.C. Roche, D.I. Smith, S.N. Thibodeau.* Dept Lab Med & Pathology, Mayo Clinic/Mayo Medical School, Rochester, MN.

Colorectal cancer (CRC) with defective DNA mismatch repair (MMR) is associated with alterations in one of several DNA MMR genes. However, the downstream functional consequences of such defects in the development of CRC are poorly understood. We previously cloned the human homolog of mouse conductin (AXIN2). AXIN2 interacts with APC, GSK3b and b-catenin and makes it a potential mutational target for colorectal cancer. We have determined its genomic structure and screened 105 CRC tumors (45 had defective MMR and the remaining 60 were MMR proficient) for mutations by DHPLC. Eleven frameshift mutations were identified and confirmed by direct sequence analysis. Significantly, mutations were identified only in CRC with defective MMR (11/45). None were detected in MMR proficient tumors (0/60; $P < 10\text{-}5$, Fishers exact test). b-catenin was shown to be accumulated in cell nuclei in 10/11 tumors with AXIN2 mutations and no APC or b-catenin mutations were detected in these tumors, demonstrating the pathologic importance of such alterations. The functional importance of these mutations was shown by the accumulation of b-catenin in the nuclei when mutant AXIN2 was transfected into normal fibroblast cells and by the strong Tcf dependent transcription upon co-transfection with mutant AXIN2 but not with wild-type AXIN2 in a Tcf reporter assay. Our data indicate that AXIN2 mutation plays an important role in CRC development by linking defective MMR to the APC pathway.

Program Nr: 41 from the 2000 ASHG Annual Meeting

Hemochromatosis gene *(HFE)* mutations are associated with an increased risk of colon cancer. *L.M. Silverman, N.J. Shaheen, R.S. Sandler, T.O. Keku, L.B. Lawrence, C.F. Martin, R. Maynard, E.M. Rohlfs.* University of North Carolina, Chapel Hill, NC.

In order to assess the association of elevated iron stores with an increased risk of colorectal neoplasia, we performed a population-based, case-control study of hemochromatosis gene *(HFE)* mutations in patients with colon cancer. Cases were 415 (41.5% African-American) patients with colon cancer from a 33 county area identified through the North Carolina Central Cancer Registry. Age-, sex, and race-matched controls (n=776, 35.7% African-American) were obtained from the same area by using Medicare records and the driver's license registry. All cases and controls completed extensive questionnaires to assess multiple environmental exposures, including dietary iron intake. Venous blood samples were obtained and assays for *(HFE)* mutations were performed on extracted DNA by polymerase chain reaction, restriction enzyme digestion, and agarose gel electrophoresis to identify both the C282Y and H63D mutations. Data were analyzed by Fisher's exact test and logistic regression. When controlled for age, sex, race, and dietary iron intake, subjects with colon cancer were significantly more likely to possess either the C282Y or H63D mutations than were controls (adjustedOR=1.40, 95% CI: 1.05-1.86). Although African-Americans are less likely than Caucasians to possess the C282Y mutation, it was a strong risk factor for colon cancer (aOR=2.31, 95% CI: 1.24-4.29). Additionally, women with any *(HFE)* mutation were also at increased risk (aOR=1.79, 95% CI: 1.18-2.72). Thus, it appears that *(HFE)* mutations are associated with an increased risk of colon cancer, with women and African-Americans having the highest risk. This increased risk may be iron-mediated or *(HFE)* may be associated with other genes that predispose to colon cancer. The work was funded by an American College of Gastroenterology Junior Faculty Development Award and R01 CA66635.

Program Nr: 42 from the 2000 ASHG Annual Meeting

Predicting the risk of gastric cancer using *H. pylori* gastritis patterns associated with family history of gastric cancer. *A.R. Sepulveda[1], L.E. Peterson[1], J. Shelton[1], D.Y. Graham[1], O. Gutierrez[2]*. 1) Department of Medicine/Gastroenterology, Baylor College of Medicine, Houston, Texas; 2) Universidad National Bogota, Colombia.

Progression of H. pylori-associated gastritis is the main pathway for gastric cancer (GC) development. Host factors may represent genetic susceptibility traits that aggregate in families with GC, and might influence the outcome of H. pylori infection. The role of family history of GC in the spectrum of H. pylori-associated histological changes of the gastric mucosa, and identification of specific patterns to predict an increased risk of GC development were investigated. Histopathological changes associated with H. pylori infection were assessed in 111 individuals with family history of GC and in 77 without. Gastric biopsies using a 12-site (5 antrum (A1-5), 6 corpus (B1-6), 1 cardia) protocol were obtained. Genta stain was used to assess H. pylori, PMN and mononuclear infiltration, lymphoid follicle (LF) load, intestinal metaplasia and atrophy using the Houston-scoring system. Individuals with positive family history of GC develop pangastritis with significantly higher ($p<0.05$) H. pylori bacterial scores in the gastric corpus while those without family history initially develop antral predominant gastritis with progressive antralization of the corpus with age. A significantly higher load of LF ($p<0.05$) was seen in the corpus of younger individuals (age<36) and in the antrum of older individuals (age 48+) with a positive family history of GC. The odds ratio for positive family history of GC based on A5LF was 4.18 (95% CI, 1.66-10.5), for B5LF was 2.82 (95% CI, 1.38-5.77), and for both was 11.81 (95% CI, 3.53-39.54) accounting for covariance. Individuals with family history of GC develop a unique histological pattern of gastritis in response to H. pylori infection. Using family history as a surrogate measure of gastric cancer risk, histological examination of lymphoid follicle load at two biopsy sites in the antrum (A5) and corpus (B5) permits the attribution of increased odds of GC risk. This information may be applicable to gastric cancer screening and surveillance in at-risk populations.

Program Nr: 43 from the 2000 ASHG Annual Meeting

Different mechanisms of PTEN inactivation in sporadic cancers are tissue-specific. *C. Eng[1], O. Gimm[1], A. Perren[1], X.P. Zhou[1], G. Mutter[2]*. 1) Human Cancer Genetics, Ohio State Univ, Columbus; 2) Brigham & Womens Hospital, Boston.

Germline PTEN mutations cause Cowden syndrome, a hamartoma syndrome with a risk of breast (BrCa), thyroid and endometrial cancers (EC). To determine if PTEN silencing can occur without genetic alteration, we performed genetic analysis and expression analysis by immunohistochemistry (mAb 6H2.1) in sporadic non-cultured Br Ca, thyroid tumors, EC, malignant melanoma (MM) and endocrine pancreatic tumors (EPT). There seemed to be 3 patterns of PTEN inactivation. One involves a first DNA mutation/deletion hit followed by epigenetic silencing (Br Ca, EC). No intragenic mutation was found among 33 sporadic invasive BrCa; 11 had hemizygous deletions, 50% of which showed no PTEN expression. In 33 EC, only 33% had deletions or mutations involving both PTEN alleles, yet 61% expressed no protein. In matched precancers, 55% had intragenic mutation while 75% had no expression. Hence, PTEN mutation is an early event initiating endometrial precancers and epigenetic PTEN silencing can precede genetic alteration in the earliest precancers. The second pattern involves both hits being epigenetic. Of 34 MM, 20% had hemizygous deletion and no intragenic mutation, yet 65% had no or decreased PTEN expression. Of the 5 with no PTEN expression, 4 had no structural abnormalities. Subcellular partitioning as a possible mechanism of PTEN inactivation is the third pattern. In 139 thyroid tumors, hemizygous deletion (25-60%) and decreased PTEN expression were associated ($p<0.01$). Decreasing PTEN expression was observed with declining degree of differentiation. Decreasing nuclear PTEN expression seemed to precede that in the cytoplasm. Among 33 EPT, only 1 had an intragenic frameshift mutation and deletion of the other allele, resulting in no PTEN expression. 8/16 informative cases had hemizygous deletion that was associated with malignant status ($P<0.05$) but not with PTEN expression. The remaining 32 EPT which expressed PTEN had a predominantly cytoplasmic expression, in contrast to normal islet cells, in which PTEN expression was nuclear. Overall, these observations suggest that specific mechanisms of inactivation predominate in a tissue-specific manner.

Program Nr: 44 from the 2000 ASHG Annual Meeting

Polyglutamines induce neurodegeneration and trans-neuronal alterations in cerebellum and retina of SCA7 transgenic mice. *G. Yvert[1], K.S. Lindenberg[2], S. Picaud[3], G.B. Landwehrmeyer[2], J-A. Sahel[3], J-L. Mandel[1]*. 1) IGBMC, Illkirch, France; 2) Dep. of Neurology, University of Ulm, Germany; 3) INSERM EMI9918, Strasbourg, France.

Among the 8 progressive neurodegenerative diseases caused by polyglutamine expansions, spinocerebellar ataxia type 7 (SCA7) is the only one to display degeneration in both brain and retina. To investigate the toxic mechanism causing SCA7, we have generated transgenic mice overexpressing full-length human ataxin-7 (harbouring 10 or 90 glutamines), either in Purkinje cells or in rod photoreceptors, by using the pcp-2 and rhodopsin promoters. In both models, expression of the mutant protein causes an observable clinical phenotype and degenerative changes in the targeted cells. We have raised antibodies against both extremities of human ataxin-7. Immunohistochemistry showed that in both mouse models, although with different time course, N-terminal immunoreactivity progressively accumulates in the nucleus of expressing cells leading to the formation of nuclear inclusions (NIs). These NIs are ubiquitinated and recruit a distinct set of chaperone/proteasome subunits. NIs are not immunoreactive for C-terminal ataxin-7 antibodies, suggesting a proteolytic processing of ataxin-7[Q90]. C-terminal immunoreactivity of mutant but not normal ataxin-7 fades with age in targeted photoreceptors. Overexpression of ataxin-7[Q90] in rods causes a severe retinal degeneration, including loss of outer segments and disorganization of the outer nuclear layer. Pathological changes appear at about 1 month of age and are correlated to an abnormal electroretinogramm response in vivo. Alterations are not limited to the targeted cell type: horizontal and rod-bipolar cells, the post-synaptic partners of rods, develop morphological changes although they do not express the mutation. Our data suggest that trans-neuronal responses are responsible for some of the neuropathological alterations seen in neurodegenerative disorders caused by polyglutamine expansions. Because retina is a suitable tissue for gene transfer and drug delivery, our model offers new opportunities to test therapeutic strategies for triplet repeat disorders.

Program Nr: 45 from the 2000 ASHG Annual Meeting

An SCA7 splice variant expressed predominantly within the CNS. *D.D. Einum[1], A.M. Clark[2], L.G. Gouw[1], L.J. Ptacek[1,2,3], Y.H. Fu[4].* 1) Dept Human Genetics; 2) Howard Hughes Medical Institute; 3) Dept Neurology; 4) Dept Neurobiology and Anatomy, Univ Utah, Salt Lake City, UT.

Translation of CAG repeat expansions encoding elongated polyglutamine tracts underlies at least 8 human diseases that involve degeneration of specific neuronal subsets despite widespread gene expression. Spinocerebellar ataxia type 7 (SCA7) is caused by polyglutamine expansion within ataxin-7, the 95 kDa product of a widely expressed gene (SCA7) on chromosome 3p, and is characterized pathologically by loss of cerebellar Purkinje cells, inferior olivary neurons of the medulla and, uniquely among the polyglutamine expansion diseases, retinal photoreceptors. A possible explanation for the restricted pattern of neurodegeneration is that polyglutamine expansion within unidentified protein isoforms with limited distributions results in increased levels of toxicity within neurons affected in the disease. As a preliminary test of this hypothesis, we searched the dbEST database at the National Center for Biotechnology Information for additional SCA7 transcripts. A single EST clone was identified that, in addition to previously reported SCA7 cDNA sequence, contains a novel 67 bp insertion. Examination of SCA7 genomic structure and RT-PCR suggest that the insertion represents an alternatively spliced exon contained within an SCA7 splice variant (SCA7.3) predicted to encode a 101 kDa ataxin-7 isoform, ataxin-7.3. Northern blot analysis reveals expression of a 5 kb transcript within the central nervous system (CNS) that is lacking in peripheral tissues. Immunoblotting demonstrates relatively high levels of ataxin-7.3 expression throughout the CNS with lower levels occurring within most peripheral tissues. Taken together, these data indicate that the gene product of an SCA7 splice variant is expressed predominantly within the CNS. Analysis of control and SCA7 tissues will shed light on the role of ataxin-7.3 in pathogenesis and may indicate whether restricted distribution of ataxin-7.3 contributes to selective neurodegeneration in SCA7.

Program Nr: 46 from the 2000 ASHG Annual Meeting

Identification of polyribosomes-associated mRNAs regulated by fragile X mental retardation protein (FMRP) using oligonucleotide microarrays. *P. Jin[1], Y. Feng[2], V. Brown[1], S.T. Warren[1]*. 1) Howard Hughes Medical Institute and Department of Biochemistry, Pediatrics and Genetics; 2) Department of Pharmacology, Emory University School of Medicine, Atlanta, GA.

Fragile X syndrome, a common form of inherited mental retardation, is mainly caused by the absence of functional FMRP, the product of FMR1 gene. FMRP is an RNA-binding protein that shuttles between the nucleus and cytoplasm and has been implicated in protein translation as it is found associated with polyribosomes. The functional significance of polyribosomal association is further solidified by the observation that FMRP point mutation, I304N, incorporates into abnormal mRNP particles and does not associate with polyribosomes. However, the downstream target genes, whose translation is directly regulated by FMRP, have not been identified yet. To accomplish this, we use oligonucleotide microarray analysis to identify the mRNAs, whose distribution is changed in the polyribosomal fraction but not at the overall transcriptional level. Using EBV-transformed human lymphoblastoid cell lines, RNA from whole cell lysates and large polyribosomal complexes were pooled to reduce individual variation, from either 5 normal males, 5 fragile X males, or an I304N patient, and subjected to microarray analysis. Compared to normal cells, more than 140 of 35,000 human genes and ESTs show significant change in the polyribosomal fraction from fragile X cells, but only 40 of them have no change at the overall transcriptional level, which suggests that distributions of these genes in polyribosomes may be regulated by FMRP. These results are further supported by finding that 15 of those 40 genes show a similar change in I304N cells. Among them is NAP-22, a cortical cytoskeleton-associated protein, which regulates nerve sprouting and synaptic growth. NAP-22 mRNA can also be co-immunoprecipitated with FMRP from mouse brain, which together suggests that NAP-22 translation is being compromised in the absence of FMRP. This study, for the first time, shows the cellular consequence in the absence of FMRP and should illuminate the physiological functions of FMRP and molecular pathogenesis of fragile X syndrome.

Program Nr: 47 from the 2000 ASHG Annual Meeting

Messenger RNAs associated with the fragile X mental retardation protein in mouse brain. *V. Brown[1], S. Ceman[1], P. Jin[1], C. Jin[1], K.D. Wilkinson[1], S.T. Warren[1,2]*. 1) Departments of Biochemistry, Genetics, and Pediatrics, Emory University School of Medicine, Atlanta, GA; 2) Howard Hughes Medical Institute, Atlanta, GA.

Fragile X syndrome, a common form of mental retardation, results from loss of FMR1 gene expression due to trinucleotide repeat expansion. FMRP is a selective RNA-binding protein which shuttles between the nucleus and the cytoplasm, and is largely cytoplasmic, associated with polyribosomes and the rough heavy ER. In order to identify those messages associated with FMRP, we developed an immunoprecipitating monoclonal antibody that does not cross-react with FMRP homologues, FXR1P and FXR2P. Immunoprecipitation (IP) from wildtype mouse brain lysates under non-denaturing conditions yields an mRNP particle composed of FMRP and at least two other proteins, FXR1P and FXR2P, as well as polyadenylated mRNA. As a control, similar IP's from the Fmr1 knockout mouse brain yields no FXR proteins and very little RNA. Using gene chips, we analyzed the mRNAs present in the IP mRNP complexes from wildtype mouse brain. Out of 19,000 gene sets screened, 300 genes were enriched in the immunoprecipitated mRNP compared to total mouse brain RNA. For 15 of these genes, including MAP1B and CAMKII, we have confirmed specific association of the mRNA with the FMRP mRNP by RT-PCR analysis of fresh IP's. We compared the distribution of the mRNAs on polysome profiles from wildtype and Fmr1 knockout mouse cells, and found significant changes in the mRNA profile for three of these genes, including Nap-22. This is the first evidence for a specific molecular biological consequence of FMRP deficiency in vivo. These data imply that FMRP regulates translation of a subset of messages in vivo. It is hypothesized that the neuronal translation of these messages will be found compromised by the absence of FMRP, thereby representing the proximal cause of fragile X syndrome.

Program Nr: 48 from the 2000 ASHG Annual Meeting

Association of acetylated histones with paternally expressed genes in the Prader-Willi/ Angelman syndrome region. *S.B. Fulmer-Smentek[1], U. Francke[1,2].* 1) HHMI; 2) Dept. of Genetics, Stanford Univ. Sch. Medicine, Stanford, CA.

Human chromosome 15q11-q13 contains a cluster of imprinted genes. Loss of the paternal or maternal allele results in Prader-Willi (PWS) or Angelman (AS) syndrome, respectively. The mechanisms of allele specific gene expression within the region are complex. Previous studies of nuclease sensitivity of SNRPN have revealed differential chromatin configuration at various sites (Schweizer, *et al.*(1999) HMG. **8**:555-66). Two paternal specific sites of nuclease sensitivity flank exon 1 of SNRPN and overlap with a region essential for the setting and maintenance of the paternal imprint. On the silent maternal allele, this region is heavily methylated. Since transcriptional silencing by DNA methylation involves histone modification by deacetylases, we hypothesized that differential histone acetylation might contribute to the allele specific expression of SNRPN and several other genes in the region. Lymphoblastoid cells from PWS, AS, or normal subjects were subjected to chromatin immunoprecipitation using antibodies against acetylated histones H3 and H4. Association of the acetylated histones with genes from the region was then assessed by multiplex PCR using GAPDH as a control. Exon 1 of SNRPN was found to be associated with acetylated histone H3 and H4 in normal and AS cells but not PWS cells, indicating that acetylated histones are present only in chromatin from the paternal allele. In contrast, intron 7 of SNRPN, which is consistently methylated only on the expressed paternal allele, was not associated with acetylated histone H3 or H4 on either allele. When other genes from the region were tested, no association with acetylated histone H3 or H4 was found for IPW, NECDIN, or MAGEL2. Paternal allele specific expression of both IPW and NECDIN was confirmed by RT-PCR in the cells studied. We conclude that differences in chromatin configuration between the two alleles of SNRPN exon 1 involves acetylation of histones H3 and H4 on the paternal allele. This allele specific histone acetylation may play a role in allele specific expression of this gene and possibly of other genes in the region.

Program Nr: 49 from the 2000 ASHG Annual Meeting

Targeted deletions in the mouse to localize *cis* elements controlling imprinting of the Prader-Willi/Angelman syndrome region. *J. Bressler, T.-F. Tsai, A.L. Beaudet.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Prader-Willi syndrome (PWS) is a neurobehavioral disorder most commonly arising from a 4 Mb deletion of paternal chromosome 15q11-q13, or from maternal uniparental disomy. PWS is characterized by mild mental retardation, gonadal hypoplasia, obsessive behavior, and hyperphagia following neonatal feeding difficulties. A cluster of paternally expressed genes including *SNRPN* has been mapped to this region, with the murine homologues localized to the syntenic region of mouse chromosome 7. The human *SNRPN* and mouse *Snrpn* genes encode two different proteins within a single transcript, with exons 1-3 encoding the SNURF protein and exons 4-10 encoding the SmN protein, a component of the spliceosome. There is evidence from human pedigrees for coordinate regulation of imprinting by a bipartite *cis*-acting imprinting center (IC) extending 100 kb centromeric of *SNRPN*. Rare PWS patients with biparental inheritance but carrying microdeletions in the portion of the IC including *SNRPN* exon 1 show inappropriate methylation of the paternal chromosome and an expression pattern characteristic of the maternal homologue. In order to define the murine IC, a 4.8 kb deletion was generated which removes *Snrpn* exon 1. Heterozygous mice inheriting the 4.8 kb deletion paternally were runted when compared with wild-type littermate controls, and about one-half of the mice carrying the deletion died before weaning. In order to assess whether the postnatal lethality and growth retardation were due to an imprinting mutation, the methylation status of the *Necdin* (*Ndn*) gene located about 1 Mb telomeric of *Snrpn* was examined by Southern blotting and revealed an alteration in methylation pattern with an increase in the fraction of methylated DNA from 50% to approximately 80%. The methylation abnormality was accompanied by a reduction in *Ndn* expression. Transcription of other paternally expressed genes including *Ipw* and *Zfp127* was not abolished when examined by RT-PCR, suggesting that the mutant mice had a partial imprinting mutation and that additional regulatory elements involved in imprinting control may lie outside the deletion boundaries.

Program Nr: 50 from the 2000 ASHG Annual Meeting

Gene conversion in the 15q imprinting center: Molecular evidence for homologous association of imprinted chromosomal domains. *B. Horsthemke[1], S. Gross[1], W. Robinson[2], K. Buiting[1]*. 1) Institut für Humangenetik, Universitätsklinikum, Essen, Germany; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada.

Using fluorescence in situ hybridization, LaSalle and Lalande (*Science* 272:725-728, 1996) observed a temporal and spatial association of the maternal and the paternal copy of the imprinted 15q11-q13 region in lymphocytes from normal individuals, but not in patients with Prader-Willi (PWS) or Angelman syndrome (AS). This observation was consistent with a "kissing model" of chromosome pairing and suggested a role for homologous association in imprint maintenance during somatic cell divisions. As pairing can entail interallelic gene conversion, we typed normal individuals as well as PWS and AS patients for a single nucleotide (C/G) polymorphism close to *SNRPN* exon 1 within the imprinting center. Allelotyping and quantitative analysis was done by fluorescence-tagged single nucleotide primer extension using the ABI SNaPshot kit and GeneScan software. Among 47 patients with uniparental heterodisomy (UPHD) and 70 patients with biparental chromosomes but uniparental DNA methylation (imprinting defects, ID), 5 maternal UPHD patients (PWS) and 8 ID patients (4 PWS and 4 AS) were heterozygous and had a balanced allelic ratio (0.5 +/- 0.1) as expected for a heterozygous state. In contrast, neither the mothers of the UPHD patients, who have the same chromosomes 15 as their affected child, nor informative parents and sibs of ID patients had a balanced ratio, but ratios of 0.02, 0.26, 0.31, 0.32, 0.69, 0.76, 0.80, 0.88, and 0.98. Among 74 control individuals, 8 were heterozygous. Of these, only three had a balanced allelic ratio, whereas the others had ratios of 0.02, 0.12, 0.23, 0.65 and 0.95. Loci outside of the imprinting center always showed balanced ratios. The skewed allelic ratios observed in normal individuals with biparental imprints, but not in patients with a uniparental imprint, are likely to reflect somatic mosaicism resulting from gene conversion events between oppositely imprinted chromosomes. Thus, in normal lymphocytes, homologous chromosomes do not only kiss, but exchange genetic information.

Program Nr: 51 from the 2000 ASHG Annual Meeting

Transcriptional profile of MS lesions reveals a complex pattern of cytokine expression. *S.E. Baranzini[1], C. Elfstrom[2], S.Y. Chang[2], R.G. Higuchi[2], J.R Oksenberg[1]*. 1) Department of Neurology, UCSF, San Francisco, CA; 2) Department of Human Genetics, Roche Molecular Systems Inc., Alameda, CA.

Multiple sclerosis (MS) is a common and severe neurological disorder, associated with an autoimmune response directed against myelin components within the central nervous system (CNS). Lymphocyte activation, extravasation and recruitment, and effector function, involves the turning on and off of a number of genes, thus triggering specific transcriptional pathways. The characterization of the transcriptome in MS lesions should provide a better understanding of the mechanisms that generate and sustain the pathogenic immune response in this disease. Here we performed transcriptional profiling of 56 relevant genes in brain specimens from 8 MS patients and 8 normal controls by kinetic RT-PCR. Statistical significance was assessed by parametric (T) and non-parametric (Rank Sum) tests. Data was further analyzed by a clustering algorithm that distributed genes and samples along a color-coded 2-D chart according to their patterns of expression. Results showed a high transcriptional activity for myelin genes MBP and MOG, however they were not differentially expressed in MS samples suggesting that remyelination is an active process also in the healthy brain. CD4 and HLA-DRA transcripts were dramatically increased in MS as compared to controls. This reveals a robust MHC class II upregulation and suggests that antigen is being presented locally to activated T cells. Analysis of cytokine and cytokine receptor genes expression showed increased levels of both the Th1 (TGF-b, RANTES, TNFaR) and Th2-molecules (IL-3, IL-5, IL-6R). Similarly both pro-inflammatory-type (CCR1, CCR5) and immunomodulatory-type (CCR4, CCR8) chemokine receptors were differentially expressed in the MS brain. This data argues against the hypothesis of strict immune polarization and suggests a complex regulation of the inflammatory response in human autoimmune demyelination.

Program Nr: 52 from the 2000 ASHG Annual Meeting

Parkin mutations and idiopathic Parkinson disease (PD). *W.K. Scott[1,2], A.R. Rogala[1,2], E. Rampersaud[1,2], J.M. Stajich[1,2], M.A. Nance[2], R.L. Watts[2], J.P. Hubble[2], B.L. Scott[1,2], J.L. Haines[2], W.C. Koller[2], M.B. Stern[2], B.C. Hiner[2], J. Jankovic[2], F.H. Allen[2], C.G. Goetz[2], G.W. Small[2], N.G. Laing[2], ion[1,2], J.M. Vance[1,2], for the Duke Center for Human Genetics/GlaxoWellcome/Deane PD Genetics Collaboration[2].* 1) Duke University Medical Center, Durham, NC; 2) Duke Center for Human Genetics/GlaxoWellcome/Deane PD Genetics Collaboration.

We have collected 864 individuals in 175 multiplex families with idiopathic PD (mean age of onset (AOO) = 60.1 ± 12.7 years) for a genomic screen (N=325 markers; 10cM grid) to identify susceptibility genes. Individuals with PD and their families were collected from 13 sites using strict consensus clinical criteria developed by participating investigators. This PD dataset is clinically similar to other adult-onset clinic-based populations (Hubble et al., Neurology 1999;52:A13). Several chromosomes with areas of interest were identified, including the region on chromosome 6 containing the Parkin gene, first described in Japanese autosomal recessive juvenile parkinsonism (AR-JP) families. Subsequent analysis demonstrated a significant genetic effect in families with ³ 1 member with onset of PD prior to age 40. AOO in these 58 families ranged from 12 to 80 years. Screening of the Parkin gene using denaturing high pressure liquid chromatography (DHPLC) has identified unique changes in a subset of these individuals. The most common change was a previously undescribed deletion of 40 bp in exon 3 found in at least 7 different families (range of AOO: 19 - 53). Analysis demonstrated that this deletion was found in both a homozygous and heterozygous form in individuals with PD. Individuals were from various geographic regions of the USA. One person with the exon 3 deletion was an initially isolated case, with age of onset at 37. These findings indicate that Parkin mutations do contribute to the common form of PD and suggest the potential use of Parkin as a diagnostic tool in familial or isolated PD with AO less than 60 years.

Program Nr: 53 from the 2000 ASHG Annual Meeting

Mouse Models of Mitochondrial Disease. *J.E. Sligh[1,2], S.E. Levy[1], P.M. Allard[1], K.G. Waymire[1], G.R. MacGregor[1], J.R. Heckenlively[3], D.C. Wallace[1].* 1) Center for Molecular Medicine, Emory University, Atlanta, GA; 2) Department of Dermatology, Emory University, Atlanta, GA; 3) Jules Stein Eye Institute, University of California, Los Angeles, CA.

Understanding of the pathophysiology of mitochondrial disorders has been limited without available animal models. We seek to generate these models using a novel approach for mitochondrial DNA (mtDNA) manipulation in mouse embryonic stem (ES) cells. We depleted female ES cells of their endogenous mitochondria using a toxin and then rescued the cells by electrofusion with cytoplasts containing mitochondria from a variety of external sources. Initial studies focused on the introduction of the mtDNA from the NZB strain of mouse containing screenable mtDNA polymorphisms. A female 129 strain ES cell line was treated with rhodamine-6G to deplete the endogenous mitochondria. The treated ES cells were rescued by electrofusion with cytoplasts containing NZB strain mtDNA, and the resulting cybrids were predominantly homoplasmic for NZB mtDNA. ES cell clones containing NZB mtDNA were injected into blastocysts to generate chimeric animals. Female chimeras were capable of transmitting the new mtDNA to their offspring which developed normally and were without overt phenotype. The introduced mtDNA was transmitted through female lineages, but never through males. This technology has now been used to transfer a mtDNA mutation in the 16S rRNA gene conferring chloramphenicol resistance (CAPr) into ES cells. Highly chimeric mice derived from the CAPr ES cells developed congenital cataracts and were found to have functional abnormalities in the retina on electroretinography. When the mutation was passed through the mouse germline, the resulting mouse pups had a severe growth retardation phenotype resulting in lethality in utero and in the perinatal period. Myopathy with disorganization and loss of contractile elements, along with a proliferation of large atypical mitochondria was observed in both skeletal and heart muscle tissue. This technology should allow for the generation of mice with a variety of other deleterious mtDNA changes that can be used for the study of basic mitochondrial biology and disease.

Program Nr: 54 from the 2000 ASHG Annual Meeting

A nuclear-mitochondrial DNA interaction affecting hearing impairment in mice. *N. Fischel-Ghodsian[1], Y. Bykhovskaya[1], O. Spirina[1], Q.Y. Zheng[2], K.R. Johnson[2].* 1) Department of Pediatrics, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, CA; 2) The Jackson Laboratory, Bar Harbor, ME.

The pathophysiological pathways and clinical expression of mitochondrial DNA (mtDNA) mutations are not well understood. This is mainly a result of the heteroplasmic nature of most pathogenic mtDNA mutations, and the absence of clinically relevant animal models with mtDNA mutations. MtDNA mutations predisposing to hearing impairment in humans are generally homoplasmic, yet some individuals with these mutations have severe hearing loss, while their maternal relatives with the identical mtDNA mutation have normal hearing. Epidemiological, biochemical, and genetic data indicate that nuclear genes are often the main determinants of these differences in phenotype. In an attempt to identify a mouse model for maternally inherited hearing loss, reciprocal backcrosses of three inbred mouse strains, A/J, NOD/LtJ and SKH2/J, with age-related hearing loss (AHL) were screened. In the (A/J x CAST/Ei) x A/J backcross, mtDNA derived from the A/J strain exerted a significant detrimental effect on hearing when compared to mtDNA from the CAST/Ei strain. This effect was not seen in the (NOD/LtJ x CAST/Ei) x NOD/LtJ and (SKH2/J x CAST/Ei) x SKH2/J backcrosses. Genotyping revealed that this effect is only seen in mice homozygous for the A/J Ahl locus on mouse chromosome 10. Sequencing of the mitochondrial genome in the three inbred strains revealed a single nucleotide insertion in the tRNA-Arg gene as the likely mediator of the mitochondrial effect. This is the first mouse model with a mtDNA mutation affecting a clinical phenotype, and provides an experimental model to dissect the pathophysiological processes connecting mtDNA mutations to hearing loss. This work was supported by NIH/NIDCD grant no. RO1DC01402 and RO1DC04092 (NFG) and contract DC62108 (KRJ).

Program Nr: 55 from the 2000 ASHG Annual Meeting

Gene expression profile of an animal model of mitochondrial myopathy using cDNA microarrays. *D.G. Murdock, S.E. Levy, D.C. Wallace.* Ctr Molec Med, Emory Univ, Atlanta, GA.

Mice deficient in the heart/muscle isoform of the adenine nucleotide translocator (ANT1) exhibit many of the hallmarks of human oxidative phosphorylation (OXPHOS) disease, including a dramatic proliferation of skeletal muscle mitochondria. Because many of the genes necessary for mitochondrial biosynthesis, OXPHOS function, and response to OXPHOS disease would be expected to be differentially expressed in ANT1 deficient mice, we have used differential display RT-PCR and cDNA microarray based expression technology in an effort to identify these genes. mRNA prepared from gastrocnemius muscle of wild type and ANT1 deficient mice was used for differential display reverse-transcription PCR reactions, or labeled and used to probe a cDNA microarray chip containing over 8000 sequence verified cDNAs. More than 150 genes have been identified as differentially expressed in ANT1 deficient skeletal muscle. While many of these differentially expressed genes are of unknown function, others can be functionally grouped. Interestingly, while all mitochondrial genes encoding OXPHOS components tested were found to be up-regulated in the mutant animals, only subsets of nuclear genes encoding OXPHOS components are differentially expressed, suggesting multiple regulatory pathways for genes that encode proteins within the same complex. Other differentially regulated genes encode products belonging to functional groups, such as groups involved in metabolism, apoptosis, signal transduction, intra- and extracellular matrix, and transcriptional regulation. These results indicate that differential expression analysis of mice deficient in ANT1 may provide new insight into the mechanism of mitochondrial biogenesis and disease, and may identify new genes previously unknown to be involved in mitochondrial function.

Program Nr: 56 from the 2000 ASHG Annual Meeting

A dominant modifier, *DFNM1*, protects seven individuals from *DFNB26* hearing impairment. *S. Riazuddin[1,2], C.M. Castelein[3], T.B. Friedman[1], A.K. Lalwani[3], A.J. Griffith[1], S. Naz[2], T.N. Smith[1], N.A. Liburd[1], M.A. Mastroianni[4], S. Riazuddin[2], E.R. Wilcox[1]*. 1) Laboratory of Molecular Genetics, NIDCD, NIH, Rockville, MD; 2) Center of Excellence in Molecular Biology, Lahore, Pakistan; 3) Laboratory of Molecular Otology, UCSF, CA; 4) Neuro-Otology Branch, NIDCD, NIH, Bethesda, MD.

We previously localized the recessive deafness locus *DFNB26* through linkage analyses to a 1.5 cM interval on chromosome 4q31. Additional members of this large consanguineous Pakistani family were enrolled and we now have data for 64 individuals, eight of whom are profoundly deaf. There is another locus *DFNM1* segregating in this family which acts as a dominant modifier of *DFNB26* that protects seven individuals homozygous for the *DFNB26* haplotype from hearing loss. These seven individuals ("escapees") come from five different branches and share a common haplotype within a 4 cM region on chromosome 1q23 as well as being homozygous for *DFNB26*. The *DFNM1* linkage region is fully contained within the reported *DFNA7* interval. None of the 8 phenotypically affected individuals in the extended family inherit the chromosome 1q23 *DFNM1* haplotype. As further evidence for a single modifier locus of *DFNB26* segregating in this family, a multipoint analysis excluded the rest of the genome to a LOD score of £ -1 for 674 markers. For *DFNB26* a maximum LOD score of 8.10 at q=0 has been obtained for the marker D4S1610 from an affected only calculation. A LOD score of 6.52 was obtained at q=0.06 for D4S1610 when all additional individuals with the unaffected phenotype were included in the analysis. A LOD score of 3.4 was obtained for the *DFNM1* by scoring the "escapees" as affected, the deaf as unaffected and scoring all others as unknown. We have carried out clinical examinations, pure tone audiometric studies and otoacoustic emission tests on six "escapees" and have found their hearing to be no different than their normal hearing siblings. Further refinement of the *DFNM1* and *DFNB26* genetic intervals should permit the identification of the responsible genes and their functions in the auditory system.

Program Nr: 57 from the 2000 ASHG Annual Meeting

Fine mapping of a nuclear modifier locus for maternally inherited deafness. *Y. Bykhovskaya[1], T. Hang[1], K. Taylor[1], R.Y.M. Tun[1], X. Estivill[2], R.A.M.S. Casano[3], K. Majamaa[4], M. Shohat[5], N. Fischel-Ghodsian[1].* 1) Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA; 2) Medical and Molecular Genetics Center, Barcelona, Spain; 3) Azienda Ospedaliera Careggi, Florence, Italy; 4) University of Oulu, Oulu, Finland; 5) Basil and Gerald Felsenstein Medical Research Center, Petah Tikva, Israel.

Maternally inherited deafness associated with the A1555G mutation in the mitochondrial 12S ribosomal RNA (rRNA) appears to require additional environmental or genetic changes for phenotypic expression. Aminoglycosides have been identified as one such environmental factor. We have recently performed a genome wide search in Arab-Israeli, Spanish and Italian families with the A1555G mutation, and no exposure to aminoglycosides, and have shown that the penetrance of the mitochondrial mutation appears to depend on additive effects of several nuclear genes. In addition, non-parametric linkage analysis possibly located one of these genes in proximity to marker D8S277, with a highly suggestive combined allele-sharing Lod score of 3.1 (Am J Hum Genet 66:1905-1910, 2000). Therefore we suggested that this region should be considered a candidate for containing the first nuclear modifier gene for a mitochondrial DNA disorder. To further investigate the significance of this finding, we have collected several additional families of Spanish, Italian and Finish origin, and typed additional markers in this region. Genotyping of all markers in the combined family set and non-parametric linkage analysis was performed. The allele-sharing Lod score increased significantly to 4.3 with the additional markers used so far, confirming the initial result. Identification of the gene in this region is hoped to provide insights into the pathophysiological pathways determining the clinical expression of the A1555G mitochondrial DNA mutation.

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Program Nr: 58 from the 2000 ASHG Annual Meeting

Mapping of a chromosomal locus for valproic acid-induced neural tube defects. *Y.W. Lundberg[1], K.A. Greer[2], J. Zhao[1], W. Xiong[1], W.J. Pavan[3], L.G. Biesecker[3], R.H. Finnell[1].* 1) Dept Pediatrics, Genetics, Univ Nebraska Medical Ctr, Omaha, NE; 2) Texas A&M University, Veterinary Anatomy and Public Health, College Station, TX 77843-4458; 3) National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892-4472.

Despite the prevalence of neural tube defects (NTDs) and the enormous economic burden they place on society, the genetic etiology of these birth defects is not well understood. We intended to dissect the genetic components underlying valproic acid (VPA)-sensitive exencephaly in the mouse. Our overall research goal is to dissect the molecular genetic mechanism that regulate sensitivity to the induction of human NTDs and to extend our knowledge of developmental processes critical to normal neural tube formation. We have utilized two highly inbred mouse strains SWV/Fnn and C57BL/6J, which differ (80% vs. 9%) in their susceptibility to VPA-caused exencephaly, to genetically map the susceptibility genes. We have generated a backcross panel consisting of 439 exencephalic fetuses (out of 1620 viable fetuses) from the backcrosses (SWVxC57)xSWV and SWVx(SWVxC57). Our backcrosses demonstrated a major fetal genetic contribution and a maternal effect. Linkage analysis of 228 of these fetuses has identified a major chromosomal locus for VPA sensitivity, and mapped the gene on mouse chromosome 7, centered on D7Mit285 ($p<2x10-12$). Our genome scan has also identified potential modifier loci around D2Mit30 ($p<0.035$) and D11Mit42 ($p<0.1$) (N=84). Next, we will pursue positional cloning and developmental study of the major gene, association study and mutation analysis using our human NTD registry with in utero VPA exposure, examining potential gene-gene interactions between this VPA gene and other genes relevant to neural tube development. Future mapping of the modifier locus (loci) may facilitate finding significant association in human NTD cases.

Program Nr: 59 from the 2000 ASHG Annual Meeting

A gene for pyridoxine-dependent epilepsy maps to chromosome 5q31. *V. Cormier-Daire[1], N. Dagoneau[1], L. Burglen[1], A. Munnich[1], O. Dulac[2].* 1) Department of Genetics and INSERM U393, Hospital Necker, Paris, France; 2) Service de Neurologie Pediatrique, Hospital Saint Vincent de Paul, Paris, France.

Pyridoxine-dependent epilepsy (PDE) is a rare autosomal recessive disorder characterized by generalized seizures responding only to pyridoxine hydrochloride (vitamin B6) in the first hours of life. The pathogenesis of PDE is unknown but it has been postulated that a reduced synthesis of gamma-aminobutyric acid (GABA) resulting from a diminished activity of the glutamic acid decarboxylase (GAD) could be responsible for the lowered threshold in PDE patients. GAD requires pyridoxal 5 phosphate (PLP) as a coenzyme for activity and an alteration in the binding of PLP to GAD has also been postulated in PDE patients. Here, we report on genetic linkage analyses in four consanguineous and one non consanguineous PDE families. The diagnosis of PDE was based on generalized seizures responding only to vitamin B6 administration. We first tested and excluded GAD1 (2q31) and GAD2 (10p23) as these genes were regarded as excellent candidates a priori. We subsequently carried out a genome wide search using microsatellite markers at an average distance of 10cM and found linkage of the disease gene to markers of chromosome 5q31.2-q31.3 (Zmax=8.43 at q=0 and Zmax=7.58 at q=0 at loci D5S2017 and D5S1972, respectively). A recombination event between loci D5S463 and D5S2090 in one family defined the distal boundary and a second recombination event between loci D5S2011 and D5S2017 in another family defined the proximal boundary of the genetic interval encompassing the PDE gene (3cM). More than 50 genes and 130 expressed sequence tags have been assigned to distal chromosome 5q. Among them, the gamma-aminobutyric acid ($GABA_A$) receptor gene and the glutamate receptor gene (GRIA1)could be regarded as candidate genes but they were excluded by our linkage data. Ongoing studies will hopefully lead to the identification of the disease causing gene.

Program Nr: 60 from the 2000 ASHG Annual Meeting

Mutations in SCO1 gene causes mitochondrial cytochrome c oxidase deficiency presenting as neonatal-onset hepatic failure and encephalopathy. *I. Valnot, S. Osmond, N. Gigarel, B. Mehaye, J.P. Bonnefont, V. Cormier-Daire, A. Munnich, P. Rustin, A. Rotig.* INSERM U393, Hosp Necker, Paris, France.

Cytochrome c oxidase (COX) catalyzes electron transfer from cytochrome c to molecular oxygen and vectorial proton pumping across the inner mitochondrial membrane. Here we report on genetic linkage analysis in a multiplex family with an isolated COX defect causing neonatal-onset hepatic failure and encephalopathy, and the subsequent identification of mutations in a nuclear gene encoding a mitochondrial assembly protein SCO1. A genome-wide search allowed us to show that the four affected sibs, but not unaffected sibs, were haplo-identical at the D17S953-D17S1796 loci (17p13.1-q11.1). This region encompasses two genes, SCO1 and COX10, encoding proteins involved in COX assembly. Mutation analyses revealed that the four patients carried heterozygous SCO1 gene mutations. One allele, inherited from the father, harbored a frame shift mutation resulting in a premature stop codon and a highly unstable mRNA, which could not be detected by RT-PCR. A second mutation (C520T) was maternally inherited and changed a highly conserved proline into a leucine in the protein (P174L). SCO1 is involved in the insertion of copper into the cytochrome c oxidase and the mutated proline residue is believed to play a crucial role in the structure of the CXXXC copper-binding domain. The mutation was absent from 103 ethnically related controls. The clinical phenotype resulting from SCO1 mutation reported here markedly differs from that of mutations in the SURF1, COX10, or SCO2 genes, despite the fact that these genes are involved in COX assembly and/or maturation. The variable degree of organ involvement might reflect variable levels of the disease gene expression in different tissues. Finally, this result emphasizes the major role of assembly genes in the pathogenesis of COX deficiency in human.

Program Nr: 61 from the 2000 ASHG Annual Meeting

A small duplication of the terminal region of chromosome 4q35 is associated with hereditary benign intraepithelial dyskeratosis. *J.M. Vance[1], R.R. Allingham[2], B. Seo[1], E. Rampersaud[1], M.L. Bembe[1], P. Challa[2], T. Parrish[1], J.R. Gilbert[1], M.A. Pericak-Vance[1,2], G.K. Klintworth[2,3].* 1) Center for Human Genetics, Duke University Medical Center, Durham, NC; 2) Dept. of Ophthalmology, DUMC, Durham, NC; 3) Dept. of Pathology, DUMC, Durham, NC.

Hereditary benign intraepithelial dyskeratosis (HBID) is an autosomal dominant disorder of cell proliferation characterized by elevated epithelial plaques on the ocular and oral mucous membranes. The vast majority of cases have been diagnosed in Native Americans of North Carolina. Onset is in early childhood and the lesions can cause visual loss. The condition has been suggested to have an seasonal environmental component that effects its expression. Using pedigree linkage analysis of two HBID families, we have localized the HBID gene to chromosome 4q35 with a lod score of 7.38. The locus lies proximal to the FSHD region. Analysis reveals that all individuals affected with HBID demonstrate the presence of three alleles for two tightly linked markers, D4S1652 and D4S2390, which map to 4q35. This strongly suggests the presence of a small duplication segregating with the disease genotype. Based on radiation hybrid studies, this duplication appears to be small, most likely 10 kb or less and is most likely involved in the etiology of the HBID phenotype.

Program Nr: 62 from the 2000 ASHG Annual Meeting

Detection of a critical interval for a familial keloid locus on chromosome 14q22-14q23 in an African-American pedigree. *K.D. Davis[1], M. Garcia[2], J.A. Phillips III[1,3], L.K. Hedges[3], J. Haines[2], J.L. Carneal[3], E. Reichenberger[4], S. Watanabe[5], B. Olsen[4], S.B. Russell[1], S.M. Williams[1,3].* 1) Dept Microbiology, Meharry Medical Col, Nashville, TN; 2) Dept Mol Physiology and Biophysics, Vanderbilt, Univ School of Medicine, Nashville, TN; 3) Dept Pediatrics, Vanderbilt Univ School of Medicine, Nashville, TN; 4) Harvard-Forsyth Dept of Oral Biology, Harvard School of Dental Med, Boston, MA; 5) Saitama Pediatric Med Center, Saitama, Japan.

Keloids are benign, collagenous tumors caused by abnormal wound healing that occur predominantly in individuals of African and Asian ancestry. While the precise molecular and biochemical dysfunctions that lead to keloid formation remain unknown, familial clustering of cases suggests that there is a strong genetic predisposition to keloid formation. We undertook a linkage study to identify the chromosomal location of the predisposing gene(s) for keloid formation for the purpose of identifying positional and functional candidate gene(s) for characterization. A genome scan was performed using one large African-American pedigree, in which nine out of 23 members form keloids. In this family the pattern of keloid inheritance is consistent with an autosomal dominant mode of inheritance. Over 170 markers on fifteen chromosomes were screened in this genome scan covering approximately 2250 cM of 3490 cM on GeneMap99. Using two-point linkage analysis (MLINK), over 500 cM were excluded prior to the finding of a significant LOD score of 3.23 with marker D14S276 on chromosome 14q22-q23. This is the maximum expected LOD score from this family, calculated with SIMLINK. Subsequent haplotype analysis of this family showed that two individuals are the products of recombination events in this region. Data from these individuals allowed the localization of the keloid locus to a 14.7 cM region between markers D14S288 and D14S274. These data indicate that keloids may be due to a single locus that resides within this 14.7 cM critical interval in some African-American kindreds with familial keloids.

Program Nr: 63 from the 2000 ASHG Annual Meeting

New candidate gene for psoriasis, HCR, shows disease association and is overexpressed in psoriatic skin. *K. Asumalahti[1], T. Laitinen[1], R. Itkonen-Vatjus[2], M-L. Lokki[3], S. Suomela[4], U. Saarialho-Kere[4], J. Kere[5]*. 1) Department of Medical Genetics, University of Helsinki; 2) Department of Dermatology, Oulu University Central Hospital; 3) Finnish Red Cross Blood Transfusion Service, Department of Tissue Typing; 4) Department of Dermatology, Helsinki University Central Hospital; 5) Finnish Genome Center, University of Helsinki, Finland.

Psoriasis is a chronic skin disorder characterized by hyperproliferation and altered differentiation of keratinocytes, angiogenesis and inflammatory leukocyte infiltrates affecting 2-3% of the Caucasoid populations. Based on association and linkage studies, a susceptibility gene for psoriasis, PSORS1, resides in chromosome 6p near the HLA-C locus. Sequencing of the region has revealed a new gene, HCR, expressed in keratinocytes and thus can be considered a good candidate gene for psoriasis. We found that HCR is highly polymorphic with 17 single nucleotide polymorphisms (SNPs) in its coding region. An association study of the new HCR polymorphisms and the previously suggested susceptibility alleles HLA-Cw*0602 and corneodesmosin allele 5 (CD*5) to psoriasis revealed a specific HCR variant associated with psoriasis susceptibility. The new susceptibility allele included two close SNPs in exon 2 both causing non-conservative amino acid changes from Threonine to Arginine (HCR*Arg-Arg). It was present in 42% (42/100) of psoriasis patients and in 19% (18/93) of population matched controls from a Finnish subpopulation (P=0.00068). A specific HCR cSNP haplotype conferred a relative risk (RR 4.8, P=0.00000018) that parallels or surpasses the risk associated with HLA-Cw*0602. Association analysis did not support CD*5 as a psoriasis susceptibility allele in our material, because it was the major allele in both groups (85-86%) and not included in an intact associated haplotype. We also found HCR to be overexpressed in keratinocytes of psoriatic lesions compared to paired samples of healthy skin with in situ hybridization. Our results suggest a potential role for HCR in the pathogenesis of psoriasis that is caused by a specific multi-cSNP variant of HCR.

Program Nr: 64 from the 2000 ASHG Annual Meeting

Mapping of the scalp-limited form of hypotrichosis simplex to chromosome 6p21.3 and exclusion of this region for the generalized form. *R.C. Betz[1], Y.-A. Lee[2], A. Bygum[3], F. Brandrup[3], A.I. Bernal[4], J. Toribio[4], J.I. Alvarez[4], G.M. Kukuk[1], H.H.W. Ibsen[3], H.B. Rasmussen[3], M. Ribera[5], M. Just[5], C. Ferrandiz[5], T.F. Wienker[2], A. Reis[2], P. Propping[1], R. Kruse[1], S. Cichon[1], M.M. Nothen[1].* 1) Institute of Human Genetics, University of Bonn, Bonn, Germany; 2) Gene Mapping Center, Max-Delbrueck Centrum, Berlin, Germany; 3) Department of Dermatology, Odense University Hospital, Odense, Denmark; 4) Department of Dermatology, University of Santiago de Compostela, Santiago de Compostela, Spain; 5) Department of Dermatology, Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

Hypotrichosis simplex is a rare, autosomal dominant form of isolated alopecia. A generalized form affecting all body hair can be clinically distinguished from a scalp-limited form. Here, we describe the localization of the first hypotrichosis simplex locus and provide evidence that the scalp-limited form maps to 6p21.3 in two Danish families and one Spanish family. We collected blood samples from 62 individuals, 27 of whom were affected. We performed a genomewide linkage analysis in the two Danish families. Haplotype analysis of recombination events localized the disease gene to a 19.4 cM region between markers D6S1663 and D6S1607. In addition, the Spanish family was tested for the same markers. Recombination events allowed us to assign the region to a 14.5 cM interval between markers D6S276 and D6S1607 with a maximum LOD score of 11.97 for all three families. Genotyping of a Spanish family with the generalized form of hypotrichosis simplex resulted in an exclusion of this locus. This suggests that the two forms of hypotrichosis simplex are due to mutations in different genes. The mapping of the gene for hypotrichosis simplex of the scalp to 6p21.3 is a first step toward identification of the gene. Relatively little is known about the processes at the molecular level that control hair growth. HSS, with no involvement of body hair, eyebrows and eyelashes may be particularly important for our understanding of the genetic, molecular and cellular pathways that regulate growth of scalp hair.

Program Nr: 65 from the 2000 ASHG Annual Meeting

Fine mapping of multiple sclerosis loci on chromosomes 5p14-p12 and 17q22-q24. *J. Saarela, H.M.F. Riise Stensland, M. Schoenberg Fejzo, D. Chen, M. Parkkonen, A. Jokiaho, A. Palotie, L. Peltonen.* Dept. of Human Genetics, UCLA School of Medicine, Los Angeles, CA 90095.

Multiple sclerosis (MS) is a neurological disorder characterized by inflammatory demyelination of the CNS. Multiple studies have suggested a genetic component to MS, and genome wide scans have revealed several putative susceptibility loci. In the genetically isolated Finnish population, four main candidate regions have been identified: the HLA-locus on chromosome 6, the MBP-locus on chromosome 18 and two relatively wide regions on chromosomes 5p12-p14 and 17q22-q24, which are syntenic to murine experimental allergic encephalomyelitis (Eae) 2 and 7 loci, respectively. To restrict these regions, we have carried out fine mapping of approximately 20 cM around D5S416 on chromosome 5, and 25 cM between markers D17S956 and D17S2182 on chromosome 17. Supportive evidence for linkage was obtained for both regions. The subset of large families originating from Southern-Bothnia, an internal isolate on the Western coast of Finland with a higher prevalence for MS, provided most information for the chromosome 17 locus. The information for the chromosome 5 locus was obtained mainly from the non-Southern-Bothnia families. Markers D5S1991 (3.64) and D17S1825 (3.42) gave the highest pairwise LODs on chromosomes 5 and 17, respectively. We have used radiation hybrid mapping, FISH, and web-based approaches to define these critical regions. Both Genehunter and Simwalk2 multipoint analysis programs further restricted the putative MS loci on chromosomes 5 and 17. Forty singleton families originating from the Southern-Bothnia region, as well as additional 119 trios from other parts of Finland, have been genotyped for the best markers, but none of the markers showed significant association with MS. We have also identified 86 intragenic SNPs for positional candidate genes on these putative MS regions. Combining the information from the microsatellite markers and the SNPs allows us to monitor efficiently for allelic association and shared haplotypes in Finnish MS alleles.

Program Nr: 66 from the 2000 ASHG Annual Meeting

A genomic screen for multiple sclerosis loci in a San Marino population supports the presence of a locus on 19q.

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Four genomic screens for multiple sclerosis (MS) have been performed (Ebers et al, 1996; Haines et al, 1996; Sawcer et al, 1996; Kuokkanen et al, 1997). A composite of 59 regions were identified as having possible involvement in MS. However, only seven regions were identified in two or more of the data sets: chromosomes (chr) 1, 2, 3 (two regions), 5, 6 and 19. Follow-up of these regions confirmed only the MHC in all four data sets. Replication difficulties may partially result from trying to detect genes of moderate effect in heterogeneous populations. To increase the power to detect susceptibility genes for MS, we employed linkage disequilibrium methods on 305 genomic screen markers from a homogeneous Republic of San Marino population. The data set includes 15 singleton families (46 sampled individuals) with a clinically mild form of MS. We performed the pedigree disequilibrium test (PDT; Martin et al.,2000). These families do not segregate the MS-associated HLA-DR2 allele and no markers in the MHC region gave significant association. Nine markers on six chrs (7, 8, 10, 19, 20, and 22) yielded a $p < 0.05$. Haplotype analyses were subsequently undertaken using Transmit (Clayton et al, 1999) for five chrs (9, 10, 18, 19, 20) with two or more adjacent markers yielding a $p<0.10$. On chr 10, one particular haplotype of D10S1213 and D10S212 was significantly over-represented in the affecteds ($p=0.006$). The most significant association, however, was detected on chr 19 with D19S589 and D19S254 where one haplotype was extremely over-represented in the affecteds ($p<0.0001$). The present results on chr 19 are particularly interesting in light of the previous linkage and association results from multiple studies, and thus further implicate chr 19q13 as harboring an MS susceptibility gene.

Program Nr: 67 from the 2000 ASHG Annual Meeting

Identification of a gene assoicated with chronic hepatitis B virus infection by genome-wide linkage analysis and family based association studies. *L. Zhang[1], A.J. Frodsham[1], S. Best[1], U. Dumpis[1], M.R. Thursz[2], H. Thomas[2], M. Graves[3], A.V.S. Hill[1]*. 1) The Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom; 2) Imperial College of Medicine, St Marys Hospital, London, United Kingdom; 3) Roche Discovery Welwyn, Roche Products Limited, 40 Broadwater Road, Welwyn Garden City, United Kingdom.

300 million people world-wide are chronically infected with hepatitis B virus (HBV). In most populations over 80% of subjects rapidly clear the infection whereas the remainder become persistent carriers. Date from a twin study and HLA association studies indicate a genetic component to the variable outcome of HBV infection. Identification of non-HLA genetic susceptibility factors provides an important new route to understanding the key pathophysiological pathways affecting the outcome of infection, and could lead to innovative therapeutic interventions. A search was therefore made through the genomewide scan in 151 Gambian families with 203 affected sib pairs using a panel of 400 highly polymorphic microsatellite markers that define a ~10cM resolution human index map. Strong evidence of linkage was found in only one region of entire genome with a single peak MLS of 3.55 (p-value = 0.000026) on chromosome 21. Further family based association studies based on polymorphisms of candidate genes in this region were carried out in the Gambian families used for the genome scan and a further set of Italian families including just over 60 affected sib pairs with the cases of persistent HBV infection. In both populations there is one highly significant ($P < 0.001$) association with a polymorphism in a candidate gene. Interestingly genes on chromosome 21 were speculated to be relevant to HBV persistence over 30 years ago following the observation that cases of trisomy 21 had a high rate of viral carriage. Reassuringly we find very similar levels of allelic association in the Italian and Gambian families indicating that this appears to be an important locus in Europeans as well as Africans.

Program Nr: 68 from the 2000 ASHG Annual Meeting

Completing the Sequence of Human Chromosome 20. *P. Deloukas.* Human Genetics, The Sanger Centre, Cambridge, UK.

The first draft of the human genome sequence is now available. With chromosomes 22 and 21 already finished the challenge ahead is to complete the remaining chromosomes for the finished reference human sequence. Completion of chromosome 20 will provide the sequence of a metacentric chromosome, enabling the study of disease loci including hematological malignancies (MPD, MDS), type 2 diabetes, familial combined hyperlipidemia and obesity. The bacterial clone map has been assembled by fingerprinting and STS content analysis. The euchromatic portion of the chromosome is in 7 contigs and clones containing telomeric and centromeric sequences that define the end of each chromosome arm have been identified. The remaining gaps (excluding the centromere) are all in the range of 30-300 kb as measured by fibre FISH, and two of them are bridged by YAC clones. The chromosome is represented in a non-redundant set of 616 clones of which 375 are finished and the remaining are in the finishing pipeline. The current output is 36.7 Mb unfinished and 36.7 Mb finished sequence, respectively (as per 1/6/2000). Computational analysis of 32.09 Mb of finished data has identified 104 known human genes, 180 putative novel genes, and 70 pseudogenes. Our findings in extending the gene annotation by experimental analysis will also be discussed. Our mapping and sequence analysis data are stored in an implementation of the database ACEDB, known as 20ace, which is available at ftp://ftp.sanger.ac.uk/pub/human/chr20/. In addition, a weekly released version of 20ace can be accessed via a WWW browser using Webace. All the links to can be found at http://www.sanger.ac.uk/HGP/Chr20.

Program Nr: 69 from the 2000 ASHG Annual Meeting

Comparison of linkage and RH with the sequence of human chromosomes 21 and 22. *T.C. Matise[1], Z. Lu[2], E.P. Sulman[2], C.J. Porter[3], A.J. Cuticchia[3], P.S. White[2].* 1) Rutgers University, Piscataway, NJ; 2) The Children's Hospital of Philadelphia, Philadelphia, PA; 3) The Hospital for Sick Children, Toronto, ON, Canada.

The availability of sequence data for human chromosome 22 represents an ultimate chromosomal map with base pair resolution, providing the opportunity to evaluate the accuracy of lower-resolution genome maps. Comparison of the predicted marker positions from various maps to the sequence-determined position allows assessment of the relationships between map distances and true physical distance, identification of cold and hot spots of recombination, prediction of regions of duplication, and identification of poorly mapped or designed markers.

A unique set of markers was identified from the Généthon, Marshfield, and CHLC linkage-based maps and the SHGC, MIT, and GeneMap RH-based maps. Using electronic PCR, these markers were queried with the chromosome 22 sequence. Of the 1079 amplimers evaluated, 148 had no match in the available chromosome 22 sequence, 32 had more than one match, and 899 had a single unique match to the sequence.

For each of the maps listed above, we identified those markers whose map positions are in agreement with sequence position and sets of markers where one or more have been misordered. The percentage of markers whose positions were consistent between the sequence and each map ranged from 58% to 100%. In order of consistency, the maps evaluated were: CHLC > GeneMap GB4 > Généthon > MIT > Marshfield > GeneMap G3 > SHGC. Most of the simple single-marker discrepancies result in a marker being placed on a map 1-8 Mb away from the observed position in the sequence. In one case a marker was placed on a map approximately 23-24 Mb away from the observed sequence position. These results show that there is wide variability in the accuracy of previously published maps, and lay the groundwork for further evaluation of this new dataset. In addition, we have identified regions of relatively increased and decreased rates of breakage. These regions generally differ between the linkage and RH maps, but there is one interesting common region of increased chromosome breakage.

Program Nr: 70 from the 2000 ASHG Annual Meeting

Characterizing Human Genomic Variation and Linkage Disequilibrium in Multiple 100kb Genomic Segments Using Large-Scale, Microarray-Based SNP Detection. *M.E. Zwick[1], D.J. Cutler[1], C.T. Yohn[1], K.P. Tobin[1], C.S. Kashuk[1], N.A. Shah[2], J.A. Warrington[2], E.E. Eichler[1], A. Chakravarti[1].* 1) Dept Genetics, Rm BRB 747B, Case Western Reserve Univ, Cleveland, OH; 2) Affymetrix, Inc., Santa Clara, CA.

Complex traits are expected to arise from the interaction of multiple segregating genetic variants that are dependent upon both the extant patterns of polymorphism and the evolutionary forces that shape this variation in natural populations. No previous study has attempted to systematically identify all unique sequence SNPs in multiple large genomic regions among a single set of samples to assess patterns of different SNP types.

We employed microarrays to identify SNPs in 40 genomic regions (32 auto-, 8 X-linked), each consisting of 50kb of unique sequence spanning a 100kb genomic region, among 40 individuals from the NIH Polymorphism Discovery Resource. Each region contained a gene with a role in brain function/development. Repetitive sequences were masked using RepeatMasker, Miropeats and ViewGene, while unique sequences were amplified using LPCR. We have screened and analyzed 22 loci and 1.1 Mb in each of 40 individuals, for a total of 80 Mb. ~3200 genomic SNPs and a four fold difference in levels of nucleotide diversity between regions (0.00052-0.00191) is observed. The mean physical distance among 993 pairs of sites (freq>5%) exhibiting significant LD (P<0.001) was 13.1kb (stdev 14.0kb, range<5 to>140kb). Tajima's D values show that all 22 regions have more rare sites than expected from a constant size neutral model (6 at P<0.05). Analysis of all 40 regions will be presented.

Extensive variation in nucleotide diversity and LD implies that average levels of these measures will be poor predictors genetic variation in any single genomic region. The excess of rare sites implies that extensive, genome-wide stratification arising from ancient human population subdivision is unlikely to exist. Our analyses explore how demography and selection have influenced patterns of genetic variation in and around genes and provide insight into choosing SNPs for genetic association studies.

Program Nr: 71 from the 2000 ASHG Annual Meeting

Long-Range Haplotypes and Linkage Disequilibrium of Single Nucleotide Polymrophisms Extend Over More Than 120 Kb in the Sequence of Human Chromosome 21. *M. Olivier, A. Aggarwal, V.I. Bustos, B.D. Foster, M.R. Levy, I. Moreno, G.A. Smick, D.R. Cox.* Dept Genetics, SHGC, Stanford Univ Sch Medicine, Palo Alto, CA.

The usefulness of single nucleotide polymorphisms (SNPs) for association studies in common human diseases is determined by their number and density, the extent of linkage disequilibrium between them, and the extent of major haplotypes over large regions of the genome. We designed 3,918 sequence-tagged sites (STSs) covering 4.2 Mb of finished sequence on chromosome 21 to study the extent of linkage disequilibrium between SNPs in different regions, and to determine the major haplotypes. On average, one STS of 400-450 bp length was designed per kb of sequence. STSs were amplified from 13 hybrid cell lines containing one independent human chromosome 21 each. SNPs were confirmed by sequencing of all 13 samples after initial detection by DHPLC. Currently, we have assessed data from 865 STSs covering approximately 900 kb of contiguous sequence on chromosome 21. We identified 353 SNPs in a total of 309,024 bp, corresponding to a detection of one SNP per 875 bp ($q=3.68x10^{-4}$). We identified a total of 138 singletons. An additional 93 SNPs (26.3% of all SNPs) were in putative mutagenic motifs (CpG dinucleotides, alpha-arrest sites, mononucleotide runs). We compared all pairs of SNPs by Fishers Exact Test and identified one region of approximately 122 kb with strong linkage disequilibrium and fewer haplotypes than expected under neutral theory. The most frequent haplotype was shared by 8 of 13 independent chromosomes, and another haplotype was identified in two additional samples. This 122 kb region does not contain any open reading frames, and its function is unknown. We are now analyzing additional data from other regions on chromosome 21 to identify other regions of strong long-range linkage disequilibrium. The use of single chromosome hybrid cell lines allows direct assessment of linkage disequilibrium and haplotype relationships in large regions of the human genome. This provides the necessary information to confidently select suitable subsets of SNPs for association studies.

Program Nr: 72 from the 2000 ASHG Annual Meeting

Integration of telomeric DNA sequences with the human reference sequence. *H.C. Riethman[1], R. Moyzis[2], E. Morse[1], L. Hu[1], D. Grady[2], H. Chi[2], S. Paul[1], Z. Xiang[1]*. 1) Wistar Inst, Philadelphia, PA; 2) Dept. of Biol Chem, College of Medicine, UC-Irvine, Irvine, CA.

DNA probes and sample sequences derived from cloned telomeric DNA regions were used to connect human telomeres with BAC contigs prepared for large-scale sequencing. Successful integration of these half-YAC-derived sequences with fingerprinted RPCI-11 BACs (either by hybridization of DNA probes with BAC libraries or by matches of single-copy sample sequences with sequence data derived from BACs) results in contiguous maps of sequence-ready DNA extending from the identified distal-most BAC contigs to specific molecular telomeres.

At present, we have connected 37 of the 41 genetically distinct telomeres with specific BAC contigs fingerprinted and assembled by the Washington University BAC Fingerprinting Project. For 33 of these telomeres, draft and/or finished sequence from production sequencing facilities exists from BACs that extend into the distal-most 50 - 300 kb telomere regions spanned by the half-YACs. This includes the four telomeres for which reference sequence already exists (16p, 4p, 7q, and 21q) and two telomeres for which reference sequence is apparently close to (but is not co-extensive with) the telomere (14q, 22q). Data will be presented summarizing our detailed half-YAC derived cosmid contig maps and sample sequencing for a group of telomeres including 1q, 2q, 7p, 9q, 10q, 11p, 13q, 15q, 16q, 17p, 18p, and 20p, delineating the extent of overlap with the flanking BAC contigs and highlighting the utility of the smaller cosmid clones from the half-YAC derived contigs in extending the sequence-ready maps and finished reference sequences to the molecular telomeres.

We have also identified by DNA hybridization and database search analysis RPCI-11 BAC contigs that contain either single or clustered sets of low-copy subtelomeric repeat sequences; we expect that many of the remaining telomeres (including the acrocentric short-arm telomeres) and many of the large, variant telomere regions known to exist will correspond to DNA from these contigs.

Program Nr: 73 from the 2000 ASHG Annual Meeting

Human gene discovery based on open reading frame-expressed sequence tags (ORESTES) in breast cancer model - First practical applications of the Human Cancer Genome Project (HCGP) / Brazil. *R.G. Correa, E. Dias Neto, S.J. De Souza, L.F.L. Reis, R.R. Brentani, A.J.G. Simpson.* Cancer Genetics Laboratory, Ludwig Institute for Cancer Research, São Paulo, SP, Brazil.

The complete human genome sequence should be available within a year. In this context, expressed sequence tags (ESTs) will be fundamental for the identification of human genes as well as for analysis of gene expression. Recently, we have developed a new approach for generating cDNA libraries using essentially arbitrary primers to construct PCR-based minilibraries from breast tumor mRNA. This preliminary study served as basis for the creation of the Human Cancer Genome Project (HCGP) in Brazil. First results are related to the generation of 6.029 ORESTES (open reading frame-expressed sequence tags) or 1.900.000 nucleotides. Bioinformatic analysis of these sequences showed that 3.350 sequences (56%) have significant similarity to known DNA and/or cDNA sequences (annotated sequences) from different organisms. From the annotated sequences, we have identified some sequences with high similarity to known genes from non human organisms, indicating the discovery of new orthologous (and paralogous) genes. Practical application of the ORESTES technique have resulted in the characterization of new human genes possibly correlated to carcinogenesis processes. In this regard, we have firstly isolated and characterized (i) a new NABC1 alternative-spliced variant (novel amplified sequence in breast carcinoma 1) which was initially correlated with an oncogenic activity on breast cancer but is down-regulated in colorectal tumors and (ii) the first human class VI semaphorin member of axon guidance molecules that is possibly correlated with nervous system development and suffers an apparent down-regulation in breast carcinomas with higher metastatic grade.

Program Nr: 74 from the 2000 ASHG Annual Meeting

A strategy for disease gene identification through NMD inhibition (GINI). *E.N. Noensie, H.C. Dietz.* HHMI and Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD.

Premature termination codons (PTCs) have been shown to cause selective degradation of mutant transcripts through the nonsense-mediated mRNA decay (NMD) pathway. We report a strategy, termed Gene Identification by NMD Inhibition (GINI), to identify genes that underlie human diseases. The strategy is based on stabilization of nonsense transcripts by pharmacologic inhibition of NMD in cell lines derived from individuals with a phenotype of interest. An optimized protocol was determined by examining the stabilization of Hexosaminidase B (HEXB) and P53 nonsense transcripts using eight drugs with multiple doses and durations of exposure. Maximal stabilization of these transcripts occurred using the translation inhibitor emetine at 100ug/ml for 10 hours. cDNA microarrays were used to evaluate expression changes induced by emetine. Wildtype transcripts upregulated by emetine were catalogued and electronically subtracted, enriching for candidates that potentially contain nonsense codons. The more conventional approach would be to compare the expression profile of control and diseased cell lines. However, by comparing a cell line to itself after treatment with emetine, the GINI strategy avoids detection of differences inherent to genetic background or compensatory changes that are only indirectly related to the disease process. GINI was tested on the colon cancer cell line HCT116 that contains a previously characterized nonsense mutation in the gene MutL Homolog 1 (MLH1). Without the use of any a priori information, a list of candidate genes was produced by GINI in which MLH1 was ranked 19th out of 7073 genes on the cDNA microarray, thus validating the strategy. In theory, GINI should be able to detect many of the human disease alleles that encode PTCs. In practice, application of this method may be limited to circumstances where nonsense alleles occur in homozygosity or hemizygosity. Currently, this is an appealing approach for the identification of disease genes underlying recessive disorders and cancer phenotypes. The power of GINI will only increase as microarray technology matures.

Program Nr: 75 from the 2000 ASHG Annual Meeting

Allelic losses due to recombination between paralogs can occur in somatic cells. *M.O. Dorschner[1], K.A. Leppig[2], M. Weaver[1], K. Stephens[1]*. 1) Division of Medical Genetics; 2) Department of Pediatrics, University of Washington, Seattle, WA.

A germline recombination event between flanking paralogous DNA segments is a common mechanism underlying contiguous gene deletion/duplication disorders, such as Charcot-Marie-Tooth 1A and neurofibromatosis (NF1) microdeletion. The role of paralogs in genomic rearrangements is extended by our identification of an NF1 microdeletion in somatic tissues. FISH and somatic cell hybrid analyses identified a 1.3 Mb deletion encompassing NF1 and contiguous loci. This deletion was present in ~50% of the patients peripheral blood cells as determined by quantitative PCR. The centromeric and telomeric breakpoints mapped to ~26 kb paralogs with ~98% nucleotide identity. Therefore, the deletion arose by paralogous recombination early in embryogenesis. This mosaic microdeletion patient had hundreds of dermal neurofibromas on all body segments at 80 years of age. This is consistent with our hypothesis, originally based on analyses of germline NF1 microdeletions, that codeletion of NF1 and a nearby gene(s) potentiates neurofibromagenesis. However, unlike the germline deletion patients who develop dermal neurofibromas in childhood, this patient had no tumors until her mid-20s. Mosaicism appears to have ameliorated the phenotype by delaying the age at onset of dermal neurofibromas. This predicts that site-specific recombination is a molecular basis for some cases of NF1 mosaicism, and possibly segmental NF1 and NF1-associated malignancies. Our data have broad implications for human disease pathogenesis. If recombination between paralogs is common during mitosis in somatic cells and confers a growth advantage, the resulting allelic changes may modify a disease phenotype, contribute to intrafamilial variability, and/or play a role in genome restructuring during neoplasia.

Program Nr: 76 from the 2000 ASHG Annual Meeting

Gene conversion and retroposition potential in recently integrated Alu elements. *P.L. Deininger[1], A.M. Roy[1], E.K. Vogel[1], M.L. Carroll[2], S.V. Nguyen[2], A.-H. Salem[2], M.A. Batzer[2].* 1) Tulane Cancer Center, SL66, Tulane Univ Medical Ctr, New Orleans, LA; 2) Dept. of Pathology, LSU Health Sciences Center, New Orleans, LA.

Alu elements comprise greater than 10% of the human genome. We have employed computational biology to analyze the human genomic DNA sequence databases to determine the impact of gene conversion on the sequence diversity of recently integrated Alu elements, and to identify Alu elements that were potentially retroposition competent. We analyzed Alu Ya5 and Alu Yb8 elements in the database representing approximately one third of the human genome and identified several small, new subfamilies. One includes the de novo Alu insertion in the NF1 gene and represents the most recent and polymorphic Alu inserts. This provides an improved selection for insertion polymorphisms that are useful for population studies. Our analysis of Alu elements containing one to four (Ya1-Ya4) of the Ya5 subfamily-specific mutations demonstrates that gene conversion contributed as much as 10-20% to the nucleotide diversity of recently integrated Alu elements. This is a surprising amount of gene conversion for such short elements. The conversion is limited to the more closely related Alu subfamilies and is directional, with the larger subfamilies converting the smaller subfamilies more often. In addition, analysis of the middle A-rich region of the different Alu Ya5 members indicates a tendency toward expansion of this region and subsequent generation of simple sequence repeats.

Program Nr: 77 from the 2000 ASHG Annual Meeting

L1 Insertion Length is Correlated with Retrotransposition Activity in a Cultured Cell Assay. *A.H. Farley, E.T. Luning Prak, H.H. Kazazian Jr.* Department of Genetics, University of Pennsylvania, School of Medicine, Philadelphia, PA.

L1 retrotransposons (L1 elements) are autonomously replicating non-long terminal repeat mobile DNA elements. Greater than 15% of the human genome is comprised of L1 elements and of these, over 95% are 5' truncated. The mechanism of 5' truncation is unknown. A number of full length human L1 elements have been cloned and assayed for retrotransposition activity in cultured cells. In this assay, different L1 elements show different levels of retrotransposition activity. We were interested in knowing whether the level of retrotransposition activity was directly correlated with the length of genomic L1 insertion. To address this question, we carried out a cultured cell assay of L1 retrotransposition using two different cloned human L1 elements, $L1_{RP}$ and L1.2. $L1_{RP}$ is approximately 50-100 times as active as L1.2 in this cultured cell assay. Following selection in G418 to recover clones which had undergone L1 retrotransposition (and were thereby rendered G418 resistant), we expanded 48 $L1_{RP}$ clones and 29 L1.2 clones. Genomic DNA from each clone was subjected to multiple rounds of PCR, using a reverse orientation primer in the neomycin resistance gene (located downstream of the L1 element) and a series of L1-specific sense-strand primers, marching progressively towards the 5' end of the L1 element. Using a primer in the very 3' end of the L1 element, 44/48 (92%) of $L1_{RP}$ clones and 27/29 (93%) of L1.2 clones gave products of the expected size. Using a primer situated 1kb from the 3 end of the L1 element, 35/48 (73%) of $L1_{RP}$ clones and 7/29 (24%) of L1.2 clones were positive. With a primer 2kb into L1, 24/48 (50%) of $L1_{RP}$ clones and 2/29 (7%) of L1.2 clones were positive. Finally, using a primer 3.5kb into the L1 element, 17/48 (36%) of $L1_{RP}$ clones and no L1.2 clones were positive. With the 3.5kb primer, the L1 insertion size is a minimum of 4.5 kb. PCR products from each genomic amplification type were verified by sequence analysis. These data suggest that L1 insertion length correlates with L1 activity in a cultured cell assay for two different human L1 elements.

Program Nr: 78 from the 2000 ASHG Annual Meeting

Assay Development of a 3-Dimensional Microarray System to Detect Mitochondrial SNPs. *J.D. Winick[1,2], J.T. Tuggle[1], A.E. Gilbreath[1], J. Xia[1], P. Gwynne[1], M. Gaskin[1], W. Klimecki[1], T. Peters[1], D. Shmulewitz[2], S. Heath[3], M.R. Bonner[1], S. Gallagher[1], J.M. Friedman[1], T.J. Raich[1]*. 1) Motorola BioChip Systems, Tempe , AZ; 2) The Rockefeller University, New York, NY; 3) Sloan Kettering Institute, New York, NY.

Motorola BioChip Systems has developed a high-throughput, single nucleotide polymorphism (SNP) analysis system which is based on microarrays of hydrophilic gel matrices. This three-dimensional microarray system offers distinct advantages over flat microarrays, including a well-characterized, covalent linkage of probes to the gel polymer and a diversity of functional groups available for biomolecule attachment. The SNP analysis system assays the DNA polymerase-mediated extension of the covalently-linked oligonucleotide probe after hybridization to a polymorphic site in a PCR-amplified target genomic DNA sample. Assays are scanned in a confocal laser slide scanner and analyzed with proprietary SNP-calling software. In order to evaluate the performance of this system, a pilot study was conducted in which the Motorola microarray SNP assay was utilized to examine haplotypes of mitochondrially-derived SNPs. In this study 400 DNA samples representing 200 distinct family lineages present in a 2000 member pedigree from the Micronesian Island of Kosrae, FSM were genotyped using 20 unscreened mitochondrial SNPs. Results of the analysis indicate the presence of 23 mitochondrial haplotypes in the Kosraen population of Micronesian origin. Call rate of a defined subset of the Kosrae population was 94% with a >99% accuracy. Data illustrating both the overall performance of this SNP genotyping system, as well as specific results from this pilot study will be presented.

Program Nr: 79 from the 2000 ASHG Annual Meeting

Comparative gene and genome mapping in pufferfish and humans. *T. Haaf[1], H. Roest Crollius[2], J. Weissenbach[2], G. Lütjens[1], B. Brunner[1], H.H. Ropers[1], F. Grützner[1].* 1) Max-Planck-Institute of Molecular Genetics, Berlin, Germany; 2) Genoscope, Evry, France.

Because of its highly compact genome, the pufferfish has become an important animal model in genome research. Although the small chromosome size renders chromosome analysis difficult, we have established both classical and molecular cytogenetics in the freshwater pufferfish *Tetraodon nigroviridis* (TNI), which is relatively easy to obtain and to keep in aquaria. TNI has an even smaller genome than the Japanese pufferfish, *Fugu rubripes* (FRU), and is used as a model for large-scale genomic sequencing (Roest Crollius *et al.*, Nature Genetics 25, 235-238, 2000). Similar to the situation in mammals, the highly compact pufferfish genome is endowed with considerable amounts of (peri)centromeric satellite DNAs and localized rRNA gene clusters. Replication banding can identify homologous chromosomes in both pufferfish species. Fluorescence in situ hybridization allows one to map single-copy sequences, i.e. the FRU *huntingtin* gene, on chromosomes of the species of origin and also on chromosomes of the heterologous pufferfish species. Therefore, mapping results obtained in either species can be integrated in one map. In order to find linkage groups which are conserved between pufferfish and humans (*Homo sapiens*, HSA), we have hybridized TNI BACs, which share sequence similarity with human genes, on pufferfish chromosomes. So far we have physically mapped the orthologous loci of >20 human X-linked genes to three different pufferfish chromosomes, TNI 1, 2, and 6, delineating three large segments of conserved chromosomal synteny. We have also established homology links for genes on HSA 4p, HSA 7q32, HSA 11p15, and the entire HSA 9. In addition, we have generated chromosome-specific DNA libraries for up to now 10 different pufferfish chromosomes by microdissection and DOP-PCR. Southern hybridization of these libraries, i.e. for TNI chromosomes 1 and 6, to gridded TNI cDNA filters and subsequent cDNA sequencing was used to identify additional genes on individual pufferfish chromosomes and to improve our first-generation homology maps.

Program Nr: 80 from the 2000 ASHG Annual Meeting

Intragenic epistasis between adaptive BRCA1 variants contribute to breast cancer risk. *G.A. Huttley[1], S.R. Wilson[2], G. Thomson[3], J.L. Hopper[4], D.J. Venter[5], S. Easteal[1].* 1) Human Genetics, John Curtin School of Medical Research, Australian National University, Canberra; 2) Centre for Mathematics and Its Applications, Australian National University, Canberra; 3) Dept. of Integrative Biology, University of California, Berkeley; 4) Centre for Genetic Epidemiology, University of Melbourne, Carlton; 5) Depts. of Pathology and Research, Peter MacCallum Cancer Institute, East Melbourne.

We have recently shown that variation at the breast cancer susceptibility gene BRCA1 has been subject to positive Darwinian selection in both humans and chimpanzees, and may be currently experiencing a selective sweep in Europeans (Huttley et al. Nature Genetics, in press, 2000). The joint involvement of BRCA1 in human disease and adaptive evolution led us to hypothesize that the incidence of some diseases may be an inextricable consequence of our adaptive evolution. If adaptive evolution is currently affecting BRCA1 variation in Europeans, interactions between the adaptive variants may influence risk of breast cancer. Several analytical approaches implicate variants at two BRCA1 polymorphic sites as adaptive. Using log-linear models to test for concordant digenic equilibria we detected differences in epistatic interactions between these sites that significantly distinguished breast cancer cases and controls (P=0.008). Consistent with our hypothesis, there was an enrichment of the haplotype comprised of both derived and ancestral amino acids in cases while haplotypes comprised solely of either derived or ancestral amino acids were enriched in controls. The results provide support for a causal relationship between adaptive modifications to BRCA1 and risk of non-familial breast cancer. They imply that the adaptive evolution of BRCA1 involves epistatic interactions between different sites within the gene. Fitness interactions among sites provides an explanation for the relatively high number of nonsynonymous substitutions that have occurred at the BRCA1 gene during human evolution.

Program Nr: 81 from the 2000 ASHG Annual Meeting

Microsatellite/RFLP haplotype analysis at the G6PD locus: Implications for the origin of malarial resistance in humans. *S.A. Tishkoff[1], S. Abbes[5], G. Argyropolous[5], G. Destro-Bisol[5], A. Drousiotou[5], G. Lefranc[5], J. Loiselet[3], A. Piro[4], M. Stoneking[5], A. Tagarelli[4], G. Tagarelli[4], E. Touma[3], S. Williams[5], T. Jenkins[2], A.G. Clark[1].* 1) Dept Biol, Penn State Univ, Univ Park, PA; 2) Dept of Hum Genet, WITS Univ, Johannesburg, S. Africa; 3) Fac of Med, Univ St Joseph, Beirut, Lebanon; 4) Inst of Exper Med and Biotech, Cosenza, Italy; 5) Others: Inst Pasteur, Tunisia; Med Univ of S. Carolina;Dept Anim/Hum Biol, "La Sapienza", Italy; The Cyprus Inst of Neuro and Genet, Cyprus; Univ of Montpellier, France; Max Planck Inst for Evol Anthro, Germany; Meharry Medical College, TN.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency, resulting from mutations within the G6PD gene on Xq28, is the most common enzymopathy of humans. The distribution and frequency of G6PD deficiency mutations is highly correlated with regions where malaria is endemic, and these mutations are thought to confer resistance to the *Plasmodium falciparum* parasite. Three novel, highly variable microsatellite polymorphisms (the most informative G6PD markers to date) have been identified within 19 kb of the G6PD gene. The frequency and distribution of alleles at these three microsatellite markers has been characterized in 12 globally diverse human populations (n=581) as well as in chimpanzee (n=13) and gorilla (n=3). These three microsatellites have been further analyzed as RFLP/microsatellite haplotypes on G6PD "A", "A-", and "Med" deficiency as well as non-deficiency chromosomes from Africa (n=317), the Middle East (n=141), and the Mediterranean (n=119) in order to reconstruct the evolutionary history of the G6PD locus and to examine the roles of mutation, migration, genetic drift, and selection on the distribution of G6PD mutations. The "A/A-" and "Med" deficiency variants exist on distinct haplotype backgrounds and are in very strong linkage disequilibrium with flanking markers out to 24 kb. These data indicate the effects of recent and strong selection at this locus and will be useful for identifying the signature of selection in genes for which the basis of functional variation that selection may act upon is unknown. Funded by NSF Sloan postdoctoral and Burroughs Wellcome Fund Career Awards to ST.

Program Nr: 82 from the 2000 ASHG Annual Meeting

Fine-Scale Mapping of Quantitative Traits Loci by Interval Mapping in Human Population. *M. Xiong, J. Zhao, L. Jin, E. Boerwinkle.* Human Gen Ctr/Houston HSC, Univ Texas, Houston, TX.

With increasing popularity of QTL mapping in humans, the need for statistical methodology for fine-scale QTL mapping becomes increasingly urgent. Although comparable advances in both methodology development and practice have been made in linkage disequilibrium fine-scale mapping for qualitative traits there is increasing recognition that statistical methods for fine-scale mapping of QTL should be developed. The ability to refine localization of the genes influencing quantitative traits depends on the number of recombination events. One way to increase the recombination events is to use historical recombination. In this report we generalize interval mapping of QTL in experimental organisms to human populations and present a general statistical framework for multipoint linkage disequilibrium mapping of QTL which can be applied to plants, animals and human populations. To implement interval mapping we develop population genetic models for haplotype frequencies. We investigate and extensively compare the statistical properties (genetic additive and dominance effects, power, sample size and support interval) of the proposed tests for multipoint linkage dsiequilibrium fine-scale mapping of QTLs under various genetic models and parameters. We show that in general the proposed models not only can be applied to isolated population, but also can be applied to admixed populations if the appropriate genetic models are emploied. The proposed method is applied to a study of loci influencing interindividual systolic blood pressure variation in a Chinese population.

Program Nr: 83 from the 2000 ASHG Annual Meeting

Detecting "stoppage" and incorporating it into a segregation analysis. *S.E. Hodge, S.L. Slager.* NY State Psychiatric Inst., Columbia Univ., New York, NY.

Segregation analysis assumes that the observed family-size distribution (FSD = distribution of number of offspring among nuclear families) is independent of the segregation ratio *p*. However, for certain serious diseases with early onset and diagnosis (e.g., autism), parents may change their family size, based on having one or more affected children, thus violating that assumption. Here we investigate "stoppage," the situation in which such parents have *fewer* children than originally planned. Following Brookfield et al. (*J Med Genet* 25: 181, 1988), we define a stoppage probability *d* that after the birth of an affected child, a family will stop having children and thus not reach their desired family size. We have demonstrated elsewhere that stoppage, if unaccounted for in the analysis, can seriously bias the results of a segregation analysis (except when ascertainment is "random," i.e., not requiring affected family members). (For example, a true *p* of .50 or .25 can be estimated as .22 or .09, respectively.) Here we examine *(a)* how to detect stoppage, and *(b)* how to correct for it. *(a)* Stoppage can be detected using a stratified Mann-Whitney U test. We show that this test is valid even in the absence of knowledge of how families were ascertained and/or knowledge of the population FSD. *(b)* We then derive the full correct likelihood as a function of *p*, *d*, and the ascertainment probability p. We show that *p* and, if necessary, *d* and/or p can be validly estimated from this likelihood if the population FSD is known. However, in the absence of knowledge of the population FSD, correcting for stoppage becomes problematic, even under single ascertainment (p®0). Moreover, we show that one solution used in the literature for truncate ascertainment (p=1) does not work, except in one extremely limited circumstance. In conclusion, stoppage, a realistic scenario for some complex diseases, can represent a serious and potentially intractable problem for segregation analysis.

Program Nr: 84 from the 2000 ASHG Annual Meeting

Patterns of linkage disequilibrium for multisite haplotypes at 14 genetic loci in human populations worldwide.

A.J. Pakstis[1], H. Zhao[1,2], J.R. Kidd[1], K.K. Kidd[1]. 1) Genetics, Yale University, New Haven, CT; 2) Epidemiology & Public Health, Yale University.

Research characterizing the extent of linkage disequilibrium (LD) across genetic loci and populations has barely begun with very few loci studied on a global sampling of populations. We are generating a unique, comprehensive dataset consisting of many genetic loci, each typed for multiple polymorphisms (SNPs, InDels, STRPs) in a large number of populations. In addition to the multisite haplotype studies we have previously published (on CD4, DM, DRD2, and PAH) we now have data on ten more loci for at least 30 to 33 populations involving over 1,765 individuals and representing all major continental regions. At present, 46 biallelic markers (SNPs and InDels) at these 14 loci (2 to 6 sites/locus spanning molecular distances up to 77 kb) have been analyzed by various LD measures including a new statistic designed to give an overall measure of non-randomness of alleles across multisite haplotypes (Zhao et al., 1999, Ann Hum Genet, 63:167-179). We find significant variation in LD among loci and among populations as well as variation in the relationship of LD and physical distance. These empiric data show that the human species is not homogeneous with respect to linkage disequilibrium--populations from different parts of the world have different amounts of linkage disequilibrium. At most loci studied so far a highly significant pattern is found for amount of LD (strongest for the multisite loci) showing Africa < Europe < East Asia < Americas. This pattern is consistent with a single recent migration out of Africa and accumulation of random drift at the front of the population expansion. The pattern also has implications for the use of LD in mapping complex disorder genes. When designing the best strategy, the population genetic complexities demonstrated by these empiric data must be taken into account. Supported in part by NIH GM57672 to KKK, JRK and GM59507 to HZ, NSF SBR9632509 to JRK.

Program Nr: 85 from the 2000 ASHG Annual Meeting

Snp Variation and Linkage Disequilibrium near the CYP1A2 Gene. *L.B. Jorde, W.S Watkins, M.J. Bamshad, D. Dunn, R. Weiss.* Human Genetics, Univ Utah, Salt Lake City, UT.

DNA sequences have been completed for a 3.4 kb promoter region of the cytochrome P450 gene, CYP1A2, in 90 members of the NIH DNA Polymorphism Discovery Resource and in 93 individuals from Africa (N=33), Asia (N=20), Europe (N=12), and the Indian subcontinent (N=28). Extensive linkage disequilibrium (LD) is found throughout the region, with no indication of a decrease in LD even for the two most distant SNPs. This result counters recent suggestions that LD may decay rapidly in the human genome. A comparison of the NIH panel and our worldwide sample reveals 14 SNPs in the former and 18 in the latter. Three SNPs are found in the NIH panel but not in the worldwide sample, while 7 SNPs are found in the worldwide sample but not in the NIH panel. All but one of these mutually exclusive SNPs have a minor allele frequency less than 1%. However, one of the polymorphisms found only in our sample has an overall minor allele frequency of 3.8% and is relatively common among Africans (10.6%). The average nucleotide diversity is 30% higher in the worldwide sample (0.00048) than in the NIH panel (0.00037). These results indicate that the NIH panel will reveal most common polymorphisms but will miss some rare ones. The panel will tend to underestimate worldwide genetic variation. A genetic structure analysis shows that Africans are the most divergent of the four populations studied. Africans and Indians have the highest levels of average nucleotide diversity (0.00057 for each); most of the diversity seen in Indians is contributed by lower caste and "tribal" populations. Less diversity is observed in Asians (0.00041) and Europeans (0.00002). The haplotypes seen in non-Africans are subsets of the more diverse group of haplotypes seen in Africans. LD for these SNPs is lowest in the African and Indian populations and highest in the European population. These findings support an African origin of modern humans. In addition, they imply that European populations will manifest LD over greater distances than will other populations and may thus require a lower density of SNPs for association-based mapping. Support: NIH GM-59290 and NSF SBR-9818215.

Program Nr: 86 from the 2000 ASHG Annual Meeting

Marker-marker linkage disequilibrium extends beyond 1 cM on chromosome 20 in Finns. *K.L. Mohlke[1], E.M. Lange[2], T. Valle[3], F.S. Collins[1], M. Boehnke[2], for the FUSION study.* 1) NHGRI, NIH, Bethesda, MD; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 3) Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland.

The power of using association analysis to map complex disease genes depends on the strength of linkage disequilibrium (LD) observed in the population and genomic region of interest. In the presence of greater LD, fewer markers need to be typed to identify ones associated with disease. Limited theoretical and experimental data report varying evidence of LD, and additional data are needed to estimate the extent and variability of LD across populations and the genome. We evaluated marker-marker LD using 43 microsatellite markers spanning chromosome 20 with an average density of 2.3 cM. We generated two-marker haplotypes for 837 individuals affected with type 2 diabetes and 386 unaffected spouse controls. All individuals are believed to be of Finnish heritage based on their grandparents' birthplaces within Finland; our sampling scheme under-represents inhabitants of western coastal Finland, an expected source of Swedish admixture. Maximum likelihood estimates were obtained for allele frequencies, by gene counting, and for haplotype frequencies, using the expectation-maximization algorithm. We used a likelihood ratio statistic to test for linkage disequilibrium. A test of homogeneity between cases and controls showed no difference in LD, as expected, allowing the 2446 chromosomes to be analyzed together. Significant ($p<0.01$) LD was observed using a likelihood ratio test in all (11/11) marker pairs within 1 cM, 78% (25/32) of pairs 1-3 cM apart and 39% (7/18) of pairs 3-4 cM apart. Consistent with chance, significant LD was observed for just over 1% (12/842) of pairs greater than 4 cM apart. Four marker pairs residing on sequence contigs and thus known to be <120 kb apart showed strongly significant LD ($p<1x10^{-10}$). These data suggest that microsatellites present at 1-cM density are sufficient to observe chromosome 20 marker-marker LD in a Finnish sample and that whole genome association studies in this population may not require ultra-high marker densities to succeed.

Program Nr: 87 from the 2000 ASHG Annual Meeting

Factors involved in the initial mutation of the fragile X CGG repeat as determined by sperm small pool-PCR.
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The fragile X syndrome is one of more than a dozen genetic diseases attributed to the amplification of a trinucleotide repeat. Despite the number of these disease loci, relatively little is known about the mechanism(s) that cause a stable allele to become unstable. Population and family studies of the fragile X CGG repeat have identified a number of factors that may play a role in repeat instability including the number of AGG interruptions, purity of the 3 end of the repeat, purity of the 5 end of the repeat, and cis-acting factors related to haplotype background. However, studies that assess whether these factors have an impact on the rate and magnitude of change of the repeat are lacking, due mainly to the lack of an appropriate model system. Therefore, in order to dissect the factors involved in the initial mutations of the CGG repeat, small pool (SP)-PCR was performed on DNA derived from sperm and blood from seven, unaffected males whose repeat sizes range from 20 to 33. In total, 211 repeat-sized variants were detected among 268,625 sperm equivalents examined and 111 repeat-sized variants were detected among 231,175 leukocyte cell equivalents examined. The overall estimated germline rate of detectable variants was 7.85x10-4, ~1.6 fold higher than the estimated somatic rate of detectable variants. Within the population of variants detected, contractions and expansions were almost equally prevalent for both sperm and leukocytes. Using the SP-PCR-derived data, regression analyses suggested that components of the repeat structure such as the number of interruptions (OR=1.678; CI: 1.165-2.417) and purity of the 3 end of the repeat (OR=1.079; CI: 1.040-1.120) are important determinants of both the rate and magnitude of change of the repeat in the germline. In contrast, elements other than repeat structure such as haplotype background (OR: 2.750; CI: 1.112-6.799) seemed to have an impact on somatic repeat instability. The factors identified for both germline and somatic instability, however, only explained a small portion of the variance (r-squared=0.1557 and 0.1571, respectively), suggesting other factors may be involved in this process.

Program Nr: 88 from the 2000 ASHG Annual Meeting

Evidence of frequent recurrent mutations at Single Nucleotide Polymorphism sites in the control region of the human mitochondrial genome. *R. Chakraborty[1], N. Wang[1], M. Kimmel[2], L. Jin[1], R. Deka[3], B. Budowle[4]*. 1) Human Genetics Ctr, Univ Texas Health Sci Ctr, Houston, TX; 2) Dept. Statistics, Rice University, Houston, TX; 3) Dept. Environmental Health, Univ. Cincinnati, Cincinnati, OH; 4) Forensic Science Research and Training Center, FBI Academy, Quantico, VA.

Allelic stability (i.e., rarity of recurrent mutations) at Single Nucleotide Polymorphism (SNP) sites has been advocated as an advantage for using SNPs in comparison with other types of polymorphisms in gene mapping, disease-gene association, and evolutionary studies. At present there is no systematic study as to how frequent are recurrent mutations at SNP sites. This research used the control region of the mtDNA genome as a paradigm to investigate this problem. From the two hypervariable segments (HV1 and HV2) of mtDNA control region, we extracted 4,159 HV1 (353 sites each) and 3,922 HV2 (284 sites each) sequences from 11 populations. In the HV1 segment 17.0% sites exhibited 3 or 4 segregating nucleotides per site (i.e., direct result of recurrent mutations); 6.7% of the HV2 sites showed the same feature. In addition, we developed a new algorithm to detect recurrent mutations at sites where only two segregating nucleotides are observed. Application of this new algorithm detected additional 52 sites in HV1 and 31 sites in HV2 segment, at each of which recurrent or reverse mutations are inferred. Thus, in total we find evidence of recurrent mutations in 31.7% of the HV1 and 17.6% of the HV2 sites sequenced, which amount to over 45% of the SNP sites at these two regions. Further, we observed a strong positive correlation ($r = 0.87$ for HV1 and $r = 0.80$ for HV2) of the likelihood recurrent mutation and gene diversity at the SNP sites of these two segments. Analysis of the distribution of base pair distances between sites showing evidence of recurrent mutations and that between the other SNP sites indicates that our results cannot be explained by recombination in the mtDNA. In summary, the above evidence demonstrates that SNP sites may not always be as stable as it is currently believed. (Research supported by US Public Health Service Research grant GM 41399 and GM 45816 from the National Institutes of Health).

Program Nr: 89 from the 2000 ASHG Annual Meeting

Haplotype inference from genotype data of random samples from populations and detection of recombination between sites. *N. Wang[1], R. Ckakraborty[1], M. Kimmel[2], L. Jin[1].* 1) Human Genetics Center, University of Texas, Houston, Houston, TX; 2) Dept. Statistics, Rice University, Houston, TX.

Haplotype inference from genotype data is a critical step of haplotype-based disease-gene association studies, and in tracing past migration history of populations. Present algorithms to derive haplotype frequencies from multilocus genotype data in random individuals make use of assumptions such as unrestricted recombination between sites, random mating in populations, or mutation-drift equilibrium. In this research we propose a novel method of haplotype inference that relaxes these assumptions. The algorithm first identifies regions of the haplotype between which signatures of past recombination events can be detected, which is subsequently used to infer haplotypes present in individuals through the use of maximum parsimony principle. We also conducted a comparative analysis of the performance of this method as compared to the others (e.g., the EMHAPFRE algorithm). Applications to several sets of multilocus genotype data indicate that number of haplotypes inferred by this method is no more than that of the others which assume free recombination and/or mutation-drift equilibrium. While the impact of these differences is relatively small in estimating nucleotide diversity, we show that phylogenetic inference of haplotype evolution can be different. Since this method does not require any assumption of population structure, genotype data from a sample of individuals of mixed ancestry can be analyzed with this new method. Using data on individuals of known haplotypes we document the accuracy of this haplotype detection method. (Research supported by US Public Health Service Research grant GM 41399 from the National Institutes of Health).

Program Nr: 90 from the 2000 ASHG Annual Meeting

Identification and characterization of markers for admixture studies of Mexican American and African American populations. *H.E. Collins[1], C.M. Phillips[1], J.L. Weber[2], R.S. Cooper[3], M.F. Seldin[1]*. 1) Rowe Program in Human Genetics, UC Davis, Davis, CA 95616; 2) Marshfield Medical Research Foundation, Marshfield, WI 54449; 3) Preventive Medicine and Epidemiology, Loyola Univ, Maywood, IL 60153.

Mapping by Admixture Linkage Disequilibrium (MALD) is a potentially powerful technique for mapping complex genetic diseases that is being developed for experimental application. The practical requirements for MALD are a set of markers spanning the genome with large differences between the parental ethnicities, and an understanding of the extent of admixture in the study populations. To this end, we have used a pooling technique to screen over 300 microsatellite and insertion deletion markers for differences between European American (EA), Amerindian (AI), African (A), African American (AA) and Mexican American (MA) populations. Approximately 20% of microsatellites and insertion deletions have differences between parental populations (delta >40%) that should provide sufficient power for MALD analyses. Estimates of admixture based on individual typing of these markers (ethnic difference markers, EDMs) are consistent with a 60% EA, 40% AI contribution to MA populations, and a 20% EA, 80% A contribution to AA populations, both of which are in agreement with anthropological and mitochondrial estimations. In addition, EDM allele frequency comparisons support the use of European Americans from California as a parental contributor to both AA and MA, Pima and Yuman speaking AI as a parental contributor to Californian MA, and Africans from Zimbabwe as a parental contributor to AA. This data suggests that differences between subpopulations of an ethnicity may be small for many markers with large inter-ethnic differences. Comparison of EDM allele distributions between AI from North and South America provide additional support for this conclusion and examination of phylogenetic relationships. Taken together, these data suggest that EDMs with large inter-population and small intra-population differences can be readily identified for MALD studies in AA and MA populations.

Program Nr: 91 from the 2000 ASHG Annual Meeting

Gene mapping across tens of centiMorgans in admixed populations. *M.W. Smith[1], J. Lautenberger[2], H.D. Shin[1], K. Washburn[2], S.J. O'Brien[2].* 1) Lab Genomic Diversity, NCI/IRSP/SAIC-Frederick, Frederick, MD; 2) Laboratory of Genomic Diversity, National Cancer Institute Frederick, MD 21702.

African American and Hispanic ethnic groups have a history of significant admixture that can serve as a genome-scan based route to disease gene discovery in a case-control design. The causes of linkage disequilibrium include of mutation, and selection along with population substructure and subsequent admixture. We empirically confirmed the theoretically predicted elevated admixture linkage disequilibrium (ALD) in African Americans across a region of 30 cM around the FY locus (Lautenberger et al., 2000 AJHG 66:969-978). A similar ongoing analysis of the GC locus has also shown elevated ALD over tens of cM. Disease gene discovery using ALD requires a map of polymorphic markers that differentiate and differences in disease allele frequencies between the founding populations. We have generated such a map by assessing allele frequencies for approximately 742 short tandem repeats in African Americans, Hispanics, European Americans and Asians and selected those markers which have high differences in composite delta, log-likelihood ratios and/or I*(2) for mapping by admixture linkage disequilibrium (MALD). Additional markers are being added to this MALD map by utilizing the rapidly growing SNP databases and the literature to a achieve a 5 10 cM scanning scale. The map will be useful for studies of diseases that have large differences in incidence between founding populations of either Hispanics or African Americans including prostate and breast cancer, diabetes, hypertension, and end-stage renal disease. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-56000.

Program Nr: 92 from the 2000 ASHG Annual Meeting

Cerebellar tremor and cerebellar cortical atrophy in older males with the fragile X premutation. *R.J. Hagerman[1], F. Tassone[2], M. Leehey[3], J. Hills[1], R. Wilson[1], W. Landau[3], J. Grigsby[2], B. Gage[4], P.J. Hagerman[2]*. 1) Fragile X Treatment & Research Center, The Children's Hospital, Denver, CO; 2) University of Colorado Health Sciences Center, Denver, CO; 3) Department of Neurology, Washington University; 4) Kaiser Permanente, Stockton, CA.

Recently a molecular phenotype has been described in males with the FMR1 premutation involving elevations in mRNA levels from 3 to 10 times normal levels. These findings are associated with a mild to moderate deficit in FMR1 protein (FMRP) levels in some of these males.

We report here 3 grandfathers with a premutation, elevated mRNA levels, and a cerebellar tremor. One individual developed a tremor at age 53 and has executive function deficits. His MRI demonstrates dilated ventricles and cerebellar atrophy. He has 98 CGG repeats, and his mRNA level is 3.38 times normal.

The second grandfather developed a vertical head tremor at age 63. MRI demonstrated dilated ventricles, cortical and cerebellar atrophy. His symptoms include memory loss and executive function deficits. He has 88 CGG repeats with a mild elevation of mRNA (2 fold above normal). He is also a carrier of spinal muscular atrophy (Werdnig-Hoffmann disease) with only 1 copy of the SMN gene.

The third case is a 63 year old grandfather with 94 CGG repeats who developed an intention tremor at age 55. He demonstrates executive function deficits, and his MRI shows cerebellar and cortical atrophy with dilated ventricles.

A decrease in size of the posterior cerebellar vermis and loss of Purkinje cells has been reported in individuals affected by fragile X syndrome, but never before in patients with the premutation. We hypothesize that a mild deficit of FMRP or an excessive elevation of mRNA may lead to late onset CNS pathology in a limited number of males with the premutation, perhaps in interaction with other mutations that impact the CNS.

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Program Nr: 93 from the 2000 ASHG Annual Meeting

Arnold-Chiari (Type I) malformation in three cases of Noonan syndrome. *B.D. Hall.* Dept Pediatrics/Genetics, Univ Kentucky Col Med, Lexington, KY.

In 1982 Ball and Peiris (J Neurol Neurosurg Psychiatry 45:753) reported the first case of Noonan syndrome associated with Arnold-Chiari, Type I (ACI), malformation. Single reports of Noonan syndrome associated with ACI by Kobayashi et al. (Jpn J Psychiatry Neurol 40:101, 86), Gabrielli et al. (Pediatr Radiol 21:16, 90), deTori et al. (Minerva Pediatr 45:347, 93), and Gabrielli et al. (Eur J Radiol 27:139, 98) followed over the next 17 years. Despite these reports and a known increased incidence of neurological problems in Noonan syndrome (Duenas et al., Southern Med J 66:193, 73, Clin Dysmorphol 6:153, 97) no systematic CNS imaging studies have been done in Noonan syndrome patients.

I present 3 sporadic, unrelated, white, females with Noonan syndrome, ages 13 5/12 years (Case 1), 9 years (Case 2), and 46 years (Case 3) who experienced headaches, dizziness, poor balance, and neck pain from 8 years, 3 years, and 43 years of age, respectively. Magnetic resonance imaging (MRI) of the CNS revealed all 3 to have ACI, which consists of extension of the cerebellar tonsils below the foramen magnum. Cases 1 and 2 recently had surgery with mixed results.

These 3 cases and the 5 literature cases indicate the need for a formal CNS imaging (MRI) study of a large series of Noonan syndrome patients to establish the true frequency of ACI.

Program Nr: 94 from the 2000 ASHG Annual Meeting

Autism in females: A genetic study. *A.L. Slaughter, N. Takahashi, R.E. Hillman, J.H. Miles.* Univ of Missouri, Columbia MO.

The skewed sex ratio in autism may be a clue to the genetic basis and heterogeneity of this complex disorder. The 214 consecutive probands who met DSM IV criteria were studied. Evaluations included morphology exams, MRIs, EEGs, neuropsychological testing and family histories. The 178 males and 36 females (4.9:1) had similar SES, ethnicity, CARS scores, seizure history, mean IQ (67 vs 65), autism recurrence risks and family history of neuropsychiatric disorders. However, the females differed from the males in important features. They had a dissimilar IQ distribution with fewer midrange scores, suggesting a bimodal distribution. The females were more often dysmorphic (25% vs 9%, $p<0.01$), microcephalic (11% vs 5%) and had structurally abnormal brains (35% vs 27%). Previous data (AJMG, 2000) demonstrated that dysmorphology, microcephaly and structural brain malformations are features that describe a complex neurodevelopmental autism phenotype. Females were almost twice as likely to have this complex phenotype (44% vs 25%, $p<0.025$). The complex phenotype males and females had lower IQs (60 vs 70 $p<0.05$), sib risks (1% vs 5%) and autism family history (11.7% vs 24.2% $p<0.05$). When the individuals with the complex phenotype were removed from consideration, the remaining normal males and females were similar in genetic variables. Behavioral evaluation, however, revealed that normal females had more pretend play (75% vs 49% $p<0.05$) and less repetitive actions (35% vs 69% $p<0.005$) than the normal boys. The results indicate that the more severe autism phenotype ascribed to females is due to a higher proportion of females who have a complex neurodevelopmental phenotype. If these individuals are removed from consideration, the remaining "normal" population of males and females are very similar. Comparison of males and females also provided information about the genetics of autism. Sib recurrence risks were identical for male and female probands (4%). This held for both the normal and complex subgroups. Brothers of probands had a higher recurrence risk (7%) than sisters (1%), independent of the sex of the proband. This is not consistent with multifactorial inheritance but suggests an innate factor in girls protecting them from expressing autism.

Program Nr: 95 from the 2000 ASHG Annual Meeting

Down syndrome associated mortality in the United States from 1983 to 1997: improved survival and paucity of cancer. *Q.H. Yang[1], S.A. Rasmussen[1], J.M. Friedman[2].* 1) BDPG/NCEH, Centers for Disease Control and Prevention, Atlanta, GA; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Down syndrome (DS), the most common identified cause of mental retardation, occurs in approximately 1 in 800 births; however, available information on DS-associated mortality is limited. In this study, we used Multiple-Cause Mortality Files compiled by the National Center for Health Statistics from US death certificates for 1983 to 1997. These files include ICD9 codes for the underlying cause of death and up to 20 contributing conditions listed on the death certificate. We selected all cases listing the code for DS anywhere on the death certificate. Among these 17,897 DS cases, mean age of death increased from 27.7 years in 1983 to 41.2 years in 1997, an average increase of 1.0 year per year studied ($p<0.05$). In contrast, mean age of death in the general population increased only 2.9 years (from 68.9 to 71.8 years) during this period. Among blacks and other races with DS, the mean age of death was significantly lower than among whites, but the improvement during the period of study was greater.

Analysis of proportionate mortality ratios (PMR) showed that DS-associated deaths were 19.0 (95% CI 17.3-20.7) times more likely to have hypothyroidism, 9.9 (95% CI 9.4-10.6) times more likely to have dementia, 9.0 (95% CI 8.8-9.3) times more likely to have congenital heart defects, and 1.5 (95% CI 1.4-1.7) times more likely to have leukemia listed on their death certificates than those who did not have DS. In contrast, malignant neoplasms other than leukemia were listed much less often than expected (PMR=0.09, 95% CI 0.08-0.10). This strikingly low PMR for malignancy other than leukemia was seen in association with DS at all ages and in both genders. This study provides population-based mortality data useful to clinicians caring for patients with Down syndrome.

Program Nr: 96 from the 2000 ASHG Annual Meeting

Association of variant alleles of mannose-binding lectin with severity of liver disease in cystic fibrosis. *J. Feingold[1], M. Gabolde[2], M. Guilloud-Bataille[1], D. Hubert[3], C. Lenaerts[1], C. Besmond[2].* 1) Inserm U155, Paris, France; 2) Inserm U 458, Paris, France; 3) Hopital Cochin, Paris, France.

Mannose-binding lectin (MBL), an important protein of the innate immune system synthesised by the liver, is involved in opsonisation and phagocytosis of micro-organisms. The MBL gene shows three major allelic variants, which are responsible for a common immunodeficiency. MBL variants are also suspected to be associated with poor evolution of hepatitis B infection. We and others have recently demonstrated that the MBL gene is a modulating gene of the respiratory involvement in cystic fibrosis (CF) patients, its major allelic variants being associated with earlier degradation of pulmonary function. Chronic liver disease is also a well-recognised complication of CF, whose precise pathogenesis remains presently unknown. Indeed there exists a substantial heterogeneity in the severity of liver disease among the group of delta F508 homozygous patients, only a minority of which developing cirrhosis, suggesting that environmental or genetic factors other than the deletion delta F508 may influence the development of CF-related liver disease. We investigated the possible modulating role of MBL in the development of CF-related cirrhosis by studying the association between the allelic variants of MBL and presence of cirrhosis in a population of 205 homogeneous delta F508 homozygous patients from adult and paediatric hospital units in France. The presence of cirrhosis was defined on the basis of hepatomegaly, abnormal liver ultrasound and portal hypertension. The patients DNA was genotyped by means of denaturing gradient gel electrophoresis. Corrected Chi-square analysis was performed. Analysis of the data shows that presence of cirrhosis in CF patients is significantly associated to a mutated MBL genotype (homozygous or compound heterozygous for MBL variants) ($p=0.006$). The modulating role of MBL in the occurrence of cirrhosis in CF patients could be explained by the fact that hepatotoxic damage due to viruses or bacteria might be increased by the immunodeficiency associated to MBL variants and might facilitate the degradation of the liver status.

Program Nr: 97 from the 2000 ASHG Annual Meeting

Homozygosity mapping of a gene locus for primary ciliary dyskinesia on chromosome 5p and identification of the heavy dynein chain *DNAH5* as a candidate gene. *H. Omran[1], K. Haeffner[1], A. Voelkel[1], J. Kuehr[1], U.P. Ketelsen[1], U.H. Ross[2], N. Konietzko[3], T. Wienker[4], M. Brandis[1], F. Hildebrandt[1]*. 1) University Childrens Hospital, Freiburg, Germany; 2) University Hospital for Ear, Nose and Throat, Freiburg, Germany; 3) Ruhrland-Klinik Essen, Germany; 4) Institute for Medical Statistics, University of Bonn, Germany.

Reduced mucociliary clearance in primary ciliary dyskinesia (PCD) causes recurrent infections of the upper and lower respiratory tract. The disease is inherited as an autosomal recessive trait. To identify a gene locus for PCD, we studied a large consanguineous family of Lebanese origin. Direct examination of the respiratory cilia revealed ciliary akinesia. Electron microscopic examination of cilia showed absence of the outer dynein arms. Two of four affected individuals exhibited a situs inversus, typical for Kartagener syndrome, due to randomization of the left/right body axis. A total genome scan with 340 highly polymorphic microsatellites was performed. We localized a new gene locus for PCD to a region of homozygosity by descent on chromosome 5p15-p14 with a parametric multipoint LOD score of Zmax = +3.51 flanked by markers D5S2095 and D5S502 within an interval of 20 cM sex-averaged genetic distance. Applying a candidate gene approach we identified the complete cDNA sequence of *DNAH5* by RACE-PCR and localized this gene within the critical disease interval of this new PCD locus. Sequence similarity studies revealed a high homology to the axonemal *Chlamydomonas* g-heavy dynein chain with conservation of functional domains. This is the first report of a complete human cDNA sequence of a gene encoding an axonemal heavy dynein chain. On the basis of the *Chlamydomonas* model for PCD this gene represents an excellent candidate for PCD.

Program Nr: 98 from the 2000 ASHG Annual Meeting

The DNAH11 (axonemal heavy chain dynein type 11) gene is mutated in one form of Primary Ciliary Dyskinesia.

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Primary Ciliary Dyskinesia (PCD) is an autosomal recessive phenotype with extensive genetic heterogeneity. Kartagener syndrome is a subphenotype of PCD characterized by situs inversus, bronchiectasis and upper respiratory tract infections. In order to define the genes responsible for PCD we have studied the DNA of a patient with immotile cilia, situs inversus and respiratory distress. This patient is remarkable because he presents 1) uniparental (paternal) disomy of chromosome 7 (patUPD7) and 2) homozygosity for F508del in the CFTR gene (Pan et al., AJHG 62:1551-5, 1998). We performed mutation analysis in all 40 known exons of the DNAH11 gene that encodes a heavy chain dynein, maps on chromosome 7, and is mutated in the mouse iv/iv model of situs inversus. A nonsense mutation (R to X) was identified in homozygosity in one of the exons coding for the motor domain of DNAH11. Electron microscopy analysis in this patient revealed normal cilia and dynein arms; this is compatible with the nonsense mutation in the middle of the motor domain of a heavy chain dynein. The mutant DNAH11 truncated protein could theoretically be incorporated in the dynein arms but it is inactive since it lacks the microtubule binding domain. We propose that the PCD phenotype of this patient is due to the nonsense DNAH11 mutation which is present in homozygosity because of the UPD7. This is the first report of a mutation in an axonemal heavy chain dynein gene causing PCD. The presence of DNAH11 mutations in other PCD families is now under study. However we only expect a small fraction of PCD patients to harbor mutations in the DNAH11 gene since our linkage analysis in 50 nuclear families did not show a significant lod score on chromosome 7p (Blouin et al., EJHG 8:109-18,2000). Lucia.Bartoloni@medecine.unige.ch

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Program Nr: 99 from the 2000 ASHG Annual Meeting

A Protein Complex Involved in Werner Syndrome Links Premature Aging to DNA Repair. *C.S. Lee[1], Y. Xue[1], X. Zhang[2], W. Wang[1]*. 1) Lab of Genetics, National Institute on Aging, National Institutes of Health, 333 Cassell Drive Suite 4000, Baltimore, MD 21224; 2) Physical and Structural chemistry, Smithkline-Beecham Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406.

Werner syndrome (WS) patients exhibit several aging features, such as skin atrophy and early graying hair, and show a premature incidence of age-related conditions including atherosclerosis, osteoporosis, type II diabetes mellitus, malignant neoplasm and cataracts. Cells from these patients have a shorter life-span than those from normal individuals, and are more similar to cells from old people. The WRN gene defective in WS has been cloned, and it belongs to the RecQ helicase family that includes at least 4 other human members. Two of them, BLM and RecQ4, are mutated in Bloom Syndrome and Rothmund-Thomson Syndrome, respectively. Inactivation of WRN orthologs in yeast also results in phenotypes of premature aging. Using an optimized immunoprecipitation method, we isolated the werner protein complex from the HeLa nuclear extracts. We report here a novel protein complex containing the WRN gene product. The complex consists of Ku70, Ku86, and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) - three proteins known to participate in double stranded DNA break (DSB) repair. Werner protein complex posesses multiple enzymatic activities, including a kinase activity that phosphorylate both p53 and RPA2, which can provoke cell cycle arrest and apoptosis. Werner protein complex from HeLa showed some different biochemical activities from the recombinant monomeric werner protein. Werner protein complex could prevent premature aging by participating in non-homologous end joining (NHEJ) repair of a special type of DSB generated by DNA replication from single strand DNA break (SSB), and by activating coordinated signaling pathways.

Program Nr: 100 from the 2000 ASHG Annual Meeting

Functional analysis of the N-terminal region of the Bloom syndrome (BS) protein BLM: evidence that the normal function of BLM depends on both its localization to the PML nuclear bodies and physical interaction with Topoisomerase IIIa**.** *P. Hu[1], S.F. Beresten[1], T. Ye[1], A.J. van Brabant[1], R. Stan[1], P.P. Pandolfi[1], F.B. Johnson[2], L. Guarente[2], N.A. Ellis[1]*. 1) Dept. of Human Genetics, Mem Sloan Kettering Cancer Ctr, New York, NY; 2) Dept. of Biology, MIT, Boston, MA.

BS is a rare, autosomal recessive, cancer-predisposing disorder that features at the cellular level increased numbers of sister-chromatid exchanges (SCEs). *BLM*, the gene mutated in BS, encodes a RecQ DNA helicase. In addition to a diffuse nucleoplasmic distribution, the BLM protein localizes to a prominent subnuclear organelle named the PML nuclear bodies (PML-NBs). Also present in the PML-NBs is Topoisomerase IIIa (Topo IIIa), with which BLM physically interacts. We have identified the regions of BLM that mediate its localization to the PML-NBs and interaction with Topo IIIa by transient transfection of a series of BLM deletion constructs tagged with GFP followed by co-immunoprecipitation and immunostaining analyses. Only the first 133 amino acids of BLM were required for its interaction with Topo IIIa, whereas BLM residues 133-438 were required for its localization to PML-NBs. Deletion of the Topo IIIa-interaction domain did not affect the localization of BLM to PML-NBs and *vice versa*, suggesting that BLM's localization to the PML-NBs and interaction with Topo IIIa are independent functions of the BLM protein. Several BS cell lines that stably express comparable levels of several key constructs were established, and the SCE numbers were compared. Whereas expressing the normal GFP-BLM can reduce the SCE numbers to nearly normal levels, expressing a GFP-BLM deletion mutant that fails to localize to PML-NBs or that fails to interact with Topo IIIa only partially corrected the high-SCE phenotype of BS cells. Deletion of both domains further diminished BLMs ability to rescue SCEs. The deletions in the N-terminus, however, did not impair the helicase activity of BLM. Our data indicate that both the proper localization of BLM in PML-NBs and its physical interaction with Topo IIIa are necessary for BLM to function normally in maintenance of genomic stability.

Program Nr: 101 from the 2000 ASHG Annual Meeting

An essential role for Fanconi anemia genes in DNA end-joining. *C. Campbell, R. Lundberg.* Dept Pharm, 6-120 Jackson Hall, Univ Minnesota Medical Sch, Minneapolis, MN.

Fanconi anemia (FA) is a heterogeneous autosomal recessive disease associated with cancer predisposition. Cultured cells from FA patients have high levels of spontaneous chromosome breaks, and are hypersensitive to DNA cross-linking agents, suggesting that FA cells may have a defect in DNA repair.

To test this hypothesis, DNA end-joining activity was measured in nuclear extracts from diploid fibroblasts belonging to FA complementation groups A, C, and D, and from several normal donors. Extracts from normal donors efficiently joined linear plasmid substrates containing 5', 3' or blunt DNA ends. In contrast, nuclear extracts from FA fibroblasts had less than 10% of the DNA end-joining activity seen in control fibroblasts. A nuclear extract prepared from retrovirally-corrected FA cells had elevated levels of DNA end-joining activity, compared to an extract from uncorrected cells.

Addition of an FA extract to a normal cell extract had no effect on the end-joining activity of the latter, suggesting that FA fibroblast extracts do not contain an inhibitor of DNA end-joining. However, when extracts from fibroblasts of FA complementation group A or C were combined with an extract from complementation group D cells, wild-type levels of DNA end-joining activity were reconstituted. Interesting, mixing extracts from FAA and FAC fibroblasts did not reconstitute DNA end-joining activity.

The FA fibroblast extracts have wild-type levels of DNA ligase IV, Xrcc4, Ku70, and Ku86. These extracts also have normal levels of DNA end-binding and DNA-dependent protein kinase activity. It thus does not appear that FA extracts are deficient in any of the previously characterized DNA end-joining proteins. Additional experimentation will be required to determine whether FA proteins directly participate in the DNA end-joining reaction, or whether their presence is needed to ensure proper levels of other, as-yet uncharacterized DNA end-joining proteins. However, these results indicate that FA genes are required for efficient DNA end-joining in fibroblast.

Program Nr: 102 from the 2000 ASHG Annual Meeting

Human L1 retrotransposition in germ cells of transgenic mice. *E.M. Ostertag[1], R.J. DeBerardinis[1], K.-S. Kim[2], G. Gerton[2], H.H. Kazazian, Jr.[1]*. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept Ob.-Gyn., Univ Pennsylvania, Philadelphia, PA.

L1 retrotransposons comprise an estimated 17% of the human genome. In addition to expanding the genome by replicating through a "copy and paste" mechanism, L1 elements cause human disease by inserting into genes. In addition, L1 elements have been implicated in mobilizing Alu elements, generating processed pseudogenes, transducing non-L1 genomic sequences, and participating in X-chromosome inactivation. To elucidate L1 biology and to estimate the frequency of L1-induced mutagenesis, we created a transgenic mouse model of retrotransposition. Our transgenes contain $L1_{RP}$, a human L1 element that inserted into the *RP2* gene of a retinitis pigmentosa patient and is currently the most active element in a cultured cell assay. We tagged $L1_{RP}$ with an EGFP retrotransposition cassette and expressed the transgenes with either the endogenous human L1 promoter or the mouse RNA polymerase II large subunit promoter (pPolII) in addition to the endogenous promoter. We created negative control transgenic lines containing an L1 with two missense mutations in the ORF1 coding region known to abolish retrotransposition. The human L1 transgene driven by its endogenous promoter was expressed in the mouse testis but not in several somatic tissues including heart, intestine, liver, and lung. Addition of pPolII permits expression in a wider tissue distribution. More specifically, human L1 transcripts driven by either promoter were detected in pachytene spermatocytes, primary spermatids, and condensed spermatids. However, RNA levels in pachytene spermatocytes from pPolII containing lines were greater than from lines containing the endogenous promoter alone. Importantly, we observed retrotransposition in fractionated male germ cells of several lines by RT-PCR. We also detected retrotransposition in at least one line by PCR of testis DNA. We did not detect retrotransposition in negative control lines. Future work to select and isolate sperm that contain new retrotransposition events may offer a novel random mutagenesis system in mouse, obviating the requirement for ES cell-based strategies.

Program Nr: 103 from the 2000 ASHG Annual Meeting

A novel chromatin protein, distantly related to histone H2A, is excluded from the inactive X chromosome and the Barr body. *B.P. Chadwick, H.F. Willard.* Dept Genetics, Center for Human Genetics, Case Western Reserve Univ, Cleveland, OH.

While it has been appreciated for more than 50 years that chromatin on the mammalian inactive X chromosome differs from that on the active X, the protein components that distinguish the two Xs in female cells are unknown. Histone variants are likely candidates for such proteins. Here, we report the identification and localization of a novel histone variant that is 48% identical to histone H2A. The protein is markedly deficient from the inactive X and from the Barr body and is thus designated Barr-d1, for Barr body-deficient protein-1. The Barr-d1 gene is located in Xq28 and is transcribed ubiquitously. To localize the protein, we transfected both Green Fluorescent Protein- and myc epitope-tagged Barr-d1 constructs into male or female human cells. Male interphase nuclei (n>50) showed a uniform distribution of Barr-d1, while female nuclei (n>200) showed a clear region of exclusion that was coincident with the Barr body, as revealed by DAPI staining and by FISH. Control experiments with histone H2B constructs revealed no such difference between male and female cells. Karyotypically abnormal human cells (47,XXX, 49,XXXXY and 49,XXXXX) showed "n-1" regions of Barr-d1 exclusion in the vast majority of nuclei. In female metaphase spreads (n>125), inactive Xs were markedly deficient in Barr-d1 staining, while the active X and the autosomes stained throughout. In double-labeling experiments, antibodies to the acetylated form of histone H4 showed a pattern of staining indistinguishable from Barr-d1 in interphase and metaphase, suggesting specific association with transcriptionally active chromatin. Chromatin fractionation studies demonstrated co-fractionation of Barr-d1 with histones, suggesting that Barr-d1 is likely to be incorporated into nucleosomes. The distribution of Barr-d1 thus distinguishes chromatin on the active and inactive Xs. Exclusion of Barr-d1 from the inactive X may be a mechanism to remodel chromatin and lock-in a transcriptionally repressed state. Determining the timing of expression and the spatial distribution of Barr-d1 in relation to other events during the early stages of X inactivation will help to elucidate the role of this protein.

Program Nr: 104 from the 2000 ASHG Annual Meeting

Identification of imprinted, tissue-specific C/D box small nucleolar RNA genes in the Prader-Willi syndrome region. *A.G Huettenhofer[1], J. Cavaille[2], K. Buiting[3], M. Kiefman[1], M. Lalande[4], C.I. Brannan[5], B. Horsthemke[3], J.P. Bachellerie[2], J. Brosius[1]*. 1) Medical Department, Experimental Pathology, Muenster, NRW, Germany; 2) Laboratoire de Biologie Moleculaire Eukaryote du CNRS, Universite Paul-Sabatier, Toulouse, France; 3) Institut fuer Humangenetik, Universitaetsklinikum, Essen, Germany; 4) Department of Genetics and Developmental Biology School of Medicine, University of Connecticut Health Center, Farmington, USA; 5) Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville, USA.

Box C/D-containing small nucleolar RNAs (snoRNAs) guide the 2-O-ribose methylation of ribosomal RNAs (rRNAs) through base pairing at each rRNA modification site. In vertebrates, known methylation guide box C/D snoRNAs are encoded as single genomic copies within introns of house-keeping genes and produced by processing of the pre-mRNA intron. So far, no tissue specific expression of snoRNAs has been observed. We have identified three novel murine C/D-box snoRNAs (MBII-13, MBII-52, MBII-85) which are expressed in brain only and are devoid of rRNA complementarity. One of them shows a 18-nt long complementarity to a mRNA for a neurotransmitter receptor. Unlike other C/D box snoRNAs, MBII-52 and MBII-85 are encoded in tandem repeats. We have isolated the human orthologues of the three snoRNAs and mapped them between the SNRPN and UBE3A genes within 15q11-q13. This region contains a number of non-protein coding genes and has been implicated in the Prader-Willi syndrome (PWS), a neurogenetic disease resulting from a deficiency of paternal gene expression. All three snoRNAs were absent in the cortex of a PWS patient and were not expressed in a mouse model for PWS in which the imprinting center (IC) is deleted. In conclusion, we have discovered a novel class of snoRNAs which display an unusual genomic organization, are expressed in a tissue-specific manner, are subject to genomic imprinting, may target mRNA and are candidates for human disease.

Program Nr: 105 from the 2000 ASHG Annual Meeting

Murine models of over and underexpression of Dyrk1A: towards an understanding of the role of DYRK1A (MNBH) in Down syndrome. *X. Estivill[1], M. Dierssen[1], V. Fotaki[1], X. Altafaj[1], C. Baamonde[2], C. Martinez-Cue[2], M. Lumbreras[2], C. Casas[1], J. Visa[1], J. Guimera[1], C. Fillat[1], J. Florez[2], M.L. Arbones[1]*. 1) Genetica Medica i Molecular, Institut Recerca Oncologica, Barcelona, Spain; 2) Physiology and Pharmacology Department, University of Cantabria, Santander, Spain.

The human DYRK1A gene is located on HSA21 within the Down syndrome (DS) critical region, and is within the region minimally deleted in HSA21-linked microcephaly. Mutations in the Drosophila mnb (minibrain) gene cause marked size reduction of brain, perturbed motor behavior and with cognitive defects. DS brains are smaller than normal with a decreased number of neurons in distinct regions. DYRK1A is expressed in regions that are affected in DS. We have generated murine models with different levels of expression of Dyrk1A. 1) While Dyrk1A (-/-) mice are not viable, Dyrk1A (+/-) are viable to adulthood and fertile, but are smaller in size and weight than control littermates, and present a marked reduction in brain size. Evaluation of neurobehavioral development revealed a delay in the achievement of physical characteristics (eyelid and ear opening), in the emergence of adult-like responses in righting and auditory Preyer's reflex and poor performance in the homing test. Adult Dyrk1A (+/-) showed reduction in horizontal and vertical activity in the open field, without gross sensory-motor alterations. Dyrk1A (+/-) presented altered learning in the Morris water maze. 2) We have also generated four lines of transgenic mice overexpressing Dyrk1A (TgDyrk1A) under the control of the SMT promoter. TgDyrk1A showed a retardation of neuromotor development with persistence of immature locomotor patterns and prolonged latencies in the homing test. Furthermore, a reduction in the latency to fall was recorded in the wire suspension test at prenatal day 15. Adult TgDyrk1A showed no differences in general behavior, learning phenotypes in the Morris water maze, motor performance in the rotarod test or circadian activity. However, decreased performance was detected in the coat-hanger test, suggesting alterations in motor coordination. Supported by CEC/BIOMED2 GENE-CT96-0054, FIS 00/0795, SAF-99-0092, PM95-0106-C02 and ERBFMBICT972278.

Program Nr: 106 from the 2000 ASHG Annual Meeting

Identification of genes which overexpression result into chromosomal instability using functional screening in yeast. *J. Flaman[1], C. Vasseur[1], N. Lemeur[1], R. Sesboué[1], P. Hieter[2], T. Frébourg[1]*. 1) INSERM EMI 9906, Faculté de Médecine et de Pharmacie, 22 boulevard Gambetta, Rouen 76183, France; 2) Dpt of Medical Genetics, Centre for Molecular Medicine and Therapeutics, University of British Columbia, 950 West 28th Avenue, Vancouver, B.C.V5Z 4H4, Canada.

Genetic instability is the hallmark of cancer. Whereas the microsatellite instability (MIN) has been shown to result from the mismatch repair genes alteration, the molecular basis of the chromosomal instability (CIN) has not been characterized so far. Somatic mutations of the human BUB1 gene, involved in the spindle checkpoint, have been found in CIN tumors, but these mutations appear to be rare. In order to identify genes which overexpression induces chromosome missegregation, we developed a functional screen in yeast *Saccharomyces cerevisiae* based on a colony color sectoring assay. In the reporter strain, the ochre *ade2-101* mutation, resulting into red pigment accumulation, is suppressed by an ochre-suppressing tRNA (SUP11), carried by a non essential artificial chromosome fragment (CF). Induction of CIN in this strain will result into loss of CF and therefore into a sectoring phenotype, characterized by the appearance of red sectors in white colonies. We screened a high-copy yeast genomic library in this reporter strain and we identified two genes, *Glc7* and *Clb5*, which overexpression affects chromosome segregation. *Glc7* encodes a serine/threonine phosphatase involved in chromosome attachment to the spindle during mitosis.*Clb5* is a B-type cyclin in yeast involved in regulation of mitosis exit. Overexpression of cyclin B2, the human homolog of Clb5, alters chromosomes segregation in yeast. This study demonstrate the power of yeast to identify candidate genes potentially involved in CIN associated to cancer.

Program Nr: 107 from the 2000 ASHG Annual Meeting

An Integrated BAC/PAC Resource for identifying chromosomal Abnormalities in Solid Tumors. *X-N. Chen[1], P. Bhattacharyya[1], Z-Y. Shi[1], S.Y. Zhao[2], L. Morsberger[3], M. Sekhon[4], J. McPherson[4], J.R. Korenberg[1]*. 1) Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center, UCLA, Los Angeles, CA; 2) The Institute for Genome Research, Rockville, MD; 3) Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD; 4) Washington University Genome Sequencing Center, St Louis, Missouri.

Cancer is a single disease and it is a hundred diseases. To understand and treat cancer, we must know the foe: its morphology, its deregulated genes and pathways, and the patterns of chromosomal alterations that are associated with malignant transformation. In order to do this, we need molecular tools to link the visible alterations of clinical disease with the underlying genes and emerging genome sequences. Our laboratory has developed such tools by creating a sequence-integrated BAC Resource for the entire genome by using high resolution FISH followed by integration with the genetic, STS and RH maps. This resource covers the human genome and contains >1,000 STS-linked BACs (Korenberg et al., 1999) and >6,000 mapped BAC clones of which 976 clones are fingerprinted and 563 are end-sequenced. These enrich the resource by integration with large-scale genome sequence, thereby defining the overlapping contigs and associated genes.

Our preliminary results support tight correspondence of DNA sequence with banding landmarks of the human genome. Further, integration of multi-site BACs suggests that unsuspected duplications target a subset of the gaps remaining in the human genome sequence. By using a combination of FISH and array technologies, this array of molecular cytogenetic markers is now used for rapid detection of cancer breakpoints and subtle aneuploidies for determining diagnostic and prognostic tumor signatures and response to treatment. All of these clones are publicly available and the integration of current resources is of the essence. As mirrored by the growth of genome information and genetic knowledge of this past century, progress will parallel collaboration. This will speed the understanding of cancers and ultimately the treatment of our aging population.

Program Nr: 108 from the 2000 ASHG Annual Meeting

Molecular analysis of non-random 8q12 deletions in Acute lymphoblastic leukemia: identification of a candidate gene involved in leukemogenesis. *B. Grandchamp[1], V. Bardet[1], L. Cattolico[2], N. Couque[1], G. Hetet[1], I. Devaux[1], S. Duprat[2], L. Gressin[3], E. Vilmer[4], H. Cave[4]*. 1) INSERM U409, Faculte Xavier Bichat, Paris, Cedex 18, France; 2) Genoscope, CNS, 91057 EVRY Evry, France; 3) CEPH, 75010 Paris, France; 4) Hopital Robert Debre,75019 Paris, France.

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood. A high resolution allelotype performed in our laboratory revealed new chromosomal sites of non-random deletions (Chambon et al, leukemia, 1998,1107-1113). We focused our work on 8q12 deletions that we found in about 4% of the patients (8 out of 205 informative cases). These deletions were of small size (less than 1Mb) in all but one patients and the common deleted region to all patients was delineated between two microsatellite markers (D8S1113 and D8S1763). This region was entirely sequenced from two overlapping BACs. The commonly deleted region (120 Kb) had a low GC content (37%), was composed of more than 50% of LINE sequences, contained no identified gene and only one single EST. Interestingly, the centromeric border of the deletions were clustered within 20Kb immediately upstream of the first exon of a HMG-1 related gene. The full length cDNA (KIAA0808) of this gene was available in public databases and all the exons but the most 3' one were found in the HTGS section of GENBANK (AC017049.3). The encoded protein presented a high similarity to TCF-1, a lymphoid transcription factor interacting with beta catenin and overexpressed in colorectal cancer. Analysis of the available mRNA from lymphoblastic cells of 2 patients with 8q12 deletions using common polymorphisms in the 3'UTR of KIAA0808 revealed a monoallelic expression of this gene. DNA analysis from the parents of one patient permitted to establish the phase with microsatellite markers within the deletion and to show that the expressed allele was on the non-deleted chromosome. Sequencing of the exons from all patients with 8q12 deletions did not show any mutation suggesting that this gene is not a classic tumor suppressor gene. Further studies are currently ongoing to better define the potential implication of KIAA0808 in leukemogenesis.

Program Nr: 109 from the 2000 ASHG Annual Meeting

Spectral Karyotyping, Comparative Genomic Hybridization and Microarray Analysis of Primary Ovarian Tumours. *J. Bayani[1,2], J.D Brenton[4], J. Karaskova[1], B. Rosen[5], J. Murphy[5], B. Zanke[3,4], J.A. Squire[1,2,3]*. 1) Dept. Cellular & Molecular Biology, Princess Margaret Hospital, Toronto Ontario, Canada; 2) Dept. Laboratory Medicine & Pathobiology, University of Toronto; 3) Dept. Medical Biophysics, University of Toronto; 4) Dept. Medical Oncology & Hematology, Dept. Experimental Therapeutics Princess Margaret Hospital; 5) Dept. Gynecologic Oncology, University Health Network, Toronto.

Ovarian cancer is the leading cause of death from gynecological malignancy. Little is known regarding the molecular and cytogenetic events that initiate and determine neoplastic disease. Recently, advanced molecular cytogenetic techniques such as Comparative Genomic Hybridization (CGH) and Spectral Karyotyping (SKY) have enabled the identification of consistent regions of amplification, gain, loss and chromosomal rearrangement in human tumours. Similarly, through advances in microarray technology, simultaneous expression profiles of thousands of genes can be determined from similar samples. In this study, we analysed 8 ovarian cancers using SKY, CGH and microarray analysis to identify consensus regions of chromosomal imbalance and chromosomal rearrangement, and to determine whether there was correlation between CGH gains and losses and gene expression. Samples were processed without prior knowledge of the histopathological classification and two-dimensional hierarchical clustering was used to order the 8 tumours into dendrograms based on similarity of expression patterns. SKY analysis identified frequent involvement of chromosomes 1, 3, 6, 8, 15, 19 and 20 in translocations. By systematically analyzing all marker chromosomes by SKY and then comparing the location of unbalanced translocations by CGH, consensus abnormal regions included frequent gains of chromosome 1, 3q, 6q, and 8q; as well as frequent losses of 3p and chromosome 4. Hierarchical clustering was able to correctly cluster 3 serous and 4 endometrioid cancers together. Using these technologies, a profile of both genomic change and corresponding expression patterns will provide a better understanding of this disease, and avenues for treatment.

Program Nr: 110 from the 2000 ASHG Annual Meeting

Cloning and characterization of FRA6E. *S.R. Denison[1], V. Shridhar[1], M.J. Ferber[1], N.A. Becker[1], G. Callahan[1], J. Lee[2], J. Lillie[2], D.I. Smith[1]*. 1) Dept. of Exp. Pathology, Mayo Clinic, Rochester, MN; 2) Millennium Predictive Medicine, Cambridge, MA.

Common fragile sites (CFS) have been hypothesized to be causally related to cancer; however, there has been a lack of conclusive evidence due to limited information pertaining to the genomic structure of these particular sites. Of the 89 currently recognized CFSs, only four, FRA3B (3p14.2), FRA7G (7q31.2), FRA7H (7q32.3), and FRA16D (16q23.2), have been cloned and characterized. Each of these sites have been associated with either a cancer-specific chromosomal rearrangement, a putative tumor suppressor gene or a region of high loss of heterozygosity (LOH) in one or more tumor types. The chromosomal band containing the fragile site FRA6E (6q26), the third most frequently observed CFS, has also been associated with high LOH in ovarian cancer. Therefore, the focus of this study was to clone and characterize FRA6E in order to compare its genomic structure to the other characterized CFSs and to determine if the gene(s) localized to this fragile region play a role in the development and/or progression of ovarian cancer. Various artificial chromosome clones localized to 6q26 were hybridized to aphidicolin-treated metaphases and a total of five clones were identified as encompassing FRA6E. FRA6E was determined to be approximately 800 kb in size, which is comparable to the size of the four other cloned CFSs. However in contrast to FRA3B, FRA7G, FRA7H and FRA16D, there are a minimum of seven genes that are located within the FRA6E region. Three of these genes (IGF2R, PLG and SLC22A3) were identified by microarray analysis as being down-regulated in primary ovarian tumors. Their status has also been analyzed by both RT-PCR and Northern Blot analysis. Additionally, LOH analysis of markers residing within FRA6E were shown to exhibit a nearly 80% loss of heterozygosity near the center of the fragile site. These data not only provide evidence to suggest that the fragility at 6q26 is important in ovarian cancer, but additional support for the hypothesis that CFSs potentially play a role in tumorigenesis and cancer progression.

Program Nr: 111 from the 2000 ASHG Annual Meeting

The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25 . *M. Ladanyi[1], M.Y. Lui[1], C.R. Antonescu[1], A. Krause-Boehm[2], A. Meindl[3], F. Mertens[4], N. Mandahl[4], H. van den Berghe[5], R. Sciot[5], P. Dal Cin[5], J.A. Bridge[2]*. 1) Memorial Sloan-Kettering Cancer Center, New York, NY; 2) University of Nebraska Medical Center, Omaha, NE; 3) Ludwig-Maximilians-Universitat, Munich, Germany; 4) University Hospital, Lund, Sweden; 5) Center for Human Genetics, University of Leuven, Belgium.

Alveolar soft part sarcoma (ASPS) is an unusual tumor with highly characteristic histopathology and ultrastructure, controversial histogenesis, and enigmatic clinical behavior. Recently, a recurrent der(17) due to a non-reciprocal t(X;17)(p11.2;q25) has been identified in this sarcoma. To define the interval containing the Xp11.2 break, we first perfomed FISH on ASPS cases using probes for OATL1 (Xp11.23) and OATL2 (Xp11.21), and the intervening genomic region. This localized the breakpoint to a 160 kb interval between cosmids U164B6 and B1125. In a previous software analysis of the complete sequence of this region, the only identifiable gene was TFE3, a transcription factor gene known to be fused to translocation partners on 1 and X in some papillary renal cell carcinomas. Southern blotting using a TFE3 genomic probe identified rearranged bands in several ASPS cases. A 5' rapid amplification of cDNA ends procedure from the 3' portion of TFE3 identified a novel sequence, designated ASPL, fused in-frame to TFE3 exon 4 (type 1 fusion) or exon 4 (type 2 fusion). Reverse transcriptase PCR using a forward primer from ASPL and a TFE3 exon 4 reverse primer detected an ASPL-TFE3 fusion transcript in all ASPS cases (11/11: 8 type 1, 3 type 2), establishing the utility of this assay in the diagnosis of ASPS. ASPL maps to chromosome 17, is ubiquitously expressed, and matches numerous ESTs but no previously characterized genes. It encodes a protein with no recognizable complex motifs. The ASPL-TFE3 fusion replaces the N-terminal portion of TFE3 by the fused ASPL sequences, while retaining the TFE3 DNA-binding domain, implicating transcriptional deregulation in the pathogenesis of this tumor, consistent with the biology of several other translocation-associated sarcomas.

Program Nr: 112 from the 2000 ASHG Annual Meeting

Quantitative DNA copy number analysis across the human genome with ~ 1 megabase resolution using array CGH. *A.M. Snijders, A.K. Hindle, R. Segraves, S. Blackwood, K. Myambo, P. Yue, X. Zhang, G. Hamilton, N. Brown, B. Huey, S. Law, J. Gray, D. Pinkel, D.G. Albertson.* Cancer Center, UCSF, San Francisco, CA.

Utilization of a microarray format for comparative genomic hybridization (array CGH) can provide copy number information with a resolution dependent on the map positions of the DNA in the array elements. Arrays of this type would be useful for scanning the genome for constitutional or somatically acquired variations in DNA copy number. We have made an array of > 2000 BAC clones from the National Cancer Institute Extramural BAC resource (http://www.nci.nih.gov/dcb/BACRESOR.htm). More will be added as this set is completed. Each of the BACs contains a mapped sequence tag, and most have been cytogenetically mapped by FISH to confirm their approximate locations and uniqueness. DNA from each BAC is robotically printed in triplicate, so that signals from replicate spots can be used to improve the measurement precision. The array is 12 mm square, and provides approximately 1 Mb resolution over the genome. The use of BACs provides sufficiently bright signals so ratio variation among the clones in a comparison of two normal genomic DNA samples is £ 10%.

Total genomic DNAs are labeled with distinct fluorochromes, typically FITC, Cy3, and Cy5, mixed with unlabeled Cot-1 DNA to suppress repetitive sequences, and hybridized overnight to the array. At least three genomic DNAs can be included in a single hybridization. Fluorescence images are acquired using a custom build CCD-based imaging system, or a commercial laser scanner. Analysis of cell lines and patient samples with known abnormalities demonstrates that the array is capable of detecting single copy gains and losses affecting individual array elements, distinguishing between heterozygous and homozygous deletions, and quantitatively determining levels of amplification. Work supported by NCI grants CA 83040, CA84118, CA80314, CA58207, and Vysis Inc.

Program Nr: 113 from the 2000 ASHG Annual Meeting

Now more than ever: The burden of genetic disease in over 4,000 consecutive pediatric hospital admissions. *S.B. Cassidy[1], J.W. Brunger[1], S. Moussavand[1], S.E. McCandless[2]*. 1) Dept Gen & Ctr for Human Gen, Case Western Reserve Univ & Univ Hosps of Cleveland, Cleveland, OH; 2) Dept Peds, Univ North Carolina, Chapel Hill, NC.

In the current medical economic environment, it is important to emphasize the significant impact of genetic disease on morbidity. Hall et al. (1978) studied patients admitted to a pediatric hospital in 1973 and found that only ~47% had a clearly non-genetic disorder. The tightening of criteria for hospital admission today, combined with the vastly increased knowledge of the genetic basis of both common and rare diseases, prompted us to reinvestigate the prevalence of genetic conditions among hospitalized children. We therefore reviewed the medical records of all admissions to Rainbow Babies and Children's Hospital in 1996. There were ~4,200 unique patients, after excluding labor & delivery and newborns. Records were evaluated for evidence of an underlying condition and were classified into categories depending on the presence or absence of a genetic component.

55% had an underlying disorder known to have a genetic component: 8.5% single gene and chromosomal, 13.9% multifactorial/polygenic birth defects (e.g., congenital heart defect); and 32.6% diseases for which there is a known genetic predisposition (e.g. asthma, cancer). 8.4% had underlying conditions with multiple known causes, often genetic (e.g., mental retardation), but the cause in the individual was unknown. 35.1% had no underlying problem (e.g., trauma in a well child, seizures following near-drowning). Studies of this type and magnitude are few, and we are not aware of similar studies in adults. Compared with the study done 25 years ago, genetic disorders are now almost twice as common, and genetic factors even more likely to underlie the medical condition, in pediatric inpatients. The burden of genetic disease on morbidity and on health care resources is thus increasingly heavy. Efforts to assure an adequate genetics workforce and educate primary care physicians are clearly justified and must take into account the fact that genetic factors contribute to over 2/3 of the conditions prompting admission to a childrens hospital.

Program Nr: 114 from the 2000 ASHG Annual Meeting

Review of inheritance patterns in nine previously unreported familial cases of Cornelia de Lange syndrome and in all reported familial cases; implications for counseling and recurrence. *K.L. Russell[1], J.E. Ming[1], L. Jackson[2], A. Bottani[3], I.D. Krantz[1]*. 1) Division of Human Genetics & Molecular Biology, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA; 2) The Division of Medical Genetics, Jefferson Medical College, Philadelphia, PA; 3) Division of Medical Genetics, Geneva University Hospital, Geneva, Switzerland.

The Cornelia de Lange syndrome (CdLS) (OMIM#122470) is a complex developmental disorder characterized by somatic and cognitive retardation, characteristic facial features, limb abnormalities, hearing loss and other organ system involvement. The majority of cases are sporadic. Males and females are equally affected. Most individuals with CdLS do not reproduce as a result of the severity of this disorder. Multiple modes of inheritance have been postulated (AD, AR, X-linked recessive, as well as the possibility of mitochondrial and an imprinting defect). Thus, recurrence-risk counseling for families is difficult. We report nine new cases of familial CdLS, four (44%) of which support autosomal dominant inheritance, three (33%) that are consistent with either autosomal recessive inheritance or germline mosaicism, and two (22%) that represent an unclear mode of inheritance. A review of the 25 reported CdLS familial cases indicates that 60% (15/25) support autosomal dominant inheritance, 28% (7/25) may represent autosomal recessive inheritance and the remaining 12% (3/25) represent an unclear pattern of inheritance. CdLS is believed to be an autosomal dominant disorder, with most cases representing new mutations in a currently unidentified gene. Although most reported families support this pattern of inheritance, several familial cases of CdLS have been identified that do not. Possible explanations for this discrepancy include gonadal mosaicism, non-penetrance, imprinting effect, and locus heterogeneity (i.e. different recessive and dominant loci).

Program Nr: 115 from the 2000 ASHG Annual Meeting

Living with Marfan Syndrome: perceptions of the Condition and its Management. *B.B. Biesecker[1], K.T. Peters[2], C.A. Francomano[1]*. 1) Medical Genetics Branch, NHGRI/NIH, Bethesda, MD; 2) Dept of Biobehavioral Health, Penn State University, University Park, PA.

We present data from 174 adults with Marfan syndrome (MS) regarding cognitive representations of the condition and use of prescribed, cardiac medications. Over 95% viewed their condition as a serious or lethal disorder. An assessment using the Illness Perception Questionnaire (Wienman, 1996) indicated that over 90% of the cohort experienced pain and/or fatigue on a regular basis. Further the subjects reported that they viewed the condition as a life-long illness, with significant health and psychosocial consequences. The group, however, reported ambivalence regarding the amount of control it has over the condition. Approximately 71% of the participants reported regularly taking a beta- or Ca++-channel blocking agent, and over 80% of these medication-takers reported that they adhered to their prescribed medication regimen. As assessed by the Beliefs about Medicines Questionnaire (Horn et al., 1996), the study cohort viewed medications as generally helpful and not overused by physicians. An assessment of the medication-takers revealed that these individuals were psychologically receptive to the use of medication for prophylactic treatment of their cardiac problems, however, they did not view their medication as essential for health. By multivariate regression medication name, duration of the medication regimen, and perceived risk for aortic root dissection were each significantly associated with an increased perception of the necessity of taking beta- or Ca++-channel blockers. Additionally, age and cardiac symptoms significantly predicted use of beta- and Ca-channel blocking agents by multivariate regression. These findings suggest that individuals with MS in this study adhere well to cardiac medication regimens, yet do not believe that the medications are essential for their health. The study cohort feels somewhat fatalistic about the health risks associated with MS. Genetic counseling ought to address beliefs about medication use and illness perception as they may have significant consequences for preventative health behaviors for individuals with MS.

Program Nr: 116 from the 2000 ASHG Annual Meeting

The Attitudes of Deaf and Hard of Hearing Individuals toward Genetic Testing of Hearing Loss. *S.J. Stern[1], K. Oelrich[2], K.S. Arnos[2], L. Murrelle[1], W.E. Nance[1], A. Pandya[1].* 1) Medical College of Virginia (MCV) at VCU, Richmond, VA; 2) Gallaudet University (GU), Washington, D.C.

Hearing loss is a common trait throughtout the world. A genetic etiology accounts for at least half of all severe to profound deafness. The recent identification of specific genes for deafness now allows genetic testing, and has made prenatal diagnosis a possibility. The attitudes of individuals with hearing loss toward these developments are not well known. We have conducted a study to assess the attitudes of deaf and hard of hearing individuals toward genetic testing, prenatal diagnosis of hearing loss, pregnancy termination, and cochlear implants. We used an anonymous written questionnaire to survey the attitudes of persons with varying degrees of hearing loss, as well as the parents of deaf children. The sample group (N=337) included members of the National Association of the Deaf, and Self Help for Hard of Hearing, Inc., as well as clinic patients at MCV, and deaf students at Gallaudet University. Respondents identified their cultural affiliation with either the Deaf community (24%), the hearing community (46%), or equally with both communities (30%). Most (34.2%) culturally Deaf respondents felt that genetic testing for hearing loss would have a negative impact on deaf people, indicated no preference for deaf or hearing children (63.3%), and were not interested in prenatal diagnosis for hearing loss (67.1%). In contrast, most (36.7%) culturally hearing respondents felt that genetic testing for hearing loss would have a positive effect on deaf people, indicated a preference for hearing children (78.5%), and would consider prenatal diagnosis for hearing loss (46.2%). Our study suggests that culturally Deaf individuals are more likely to have a negative view of genetic and prenatal testing for hearing loss than culturally hearing individuals. Awareness of these attitudes should allow caregivers to provide more effective genetic counseling, and may increase utilization of genetic services and participation in research by members of the Deaf community.

Program Nr: 117 from the 2000 ASHG Annual Meeting

Genetic Counseling Services at a Minority Serving Institution: Addressing the Needs of High Risk, Underserved Populations . *I.S. Mittman, B.F. Harrison, V.E. Headings, R.F. Murray.* College of Medicine, Howard University, Washington, DC.

Howard University Hospital (HUH) serves a high-risk patient population with respect to genetic conditions, predominantly, African-Americans, and immigrants from Africa, the Caribbean Islands and Latin America. The HUH patient population experiences significant barriers to medical services including financial, educational, and physical difficulties in attending medical appointments.

In order to increase access to genetic counseling services at HUH, a genetic counseling program was put together with support from the federal Maternal and Child Health Bureau. Two full time genetic counselors with expertise in serving culturally diverse populations were recruited. The project's staff launched an extensive effort to educate Howard University's providers about the new services. In-service training sessions, grand rounds and seminars were provided with a focus on medical genetics services.

In order to make services user-friendly for patients, the following interventions took place: Patients were seen by the genetic counselor while present for another medical visit. Follow-up visits were then scheduled if necessary after making the initial contact.

These interventions afforded a substantial increase in patients seen over the three-year period and yielded a much higher show-up rate as compared to previous years. This can be demonstrated by the fact that within the first grant year, the number of patient contacts increased three-fold from the period prior to the project's initiation. Significantly, referrals were received from a host of new referring departments and services.

Providers serving underserved patient populations need to take into account patients' daily realities and their unique needs. Service logistic modifications that are relatively easily adapted can assure a much-needed equity in access to genetic care.

Program Nr: 118 from the 2000 ASHG Annual Meeting

Integrating genetic counseling into the care of long-term survivors of childhood cancer: A model program. *K.A. Schneider[1], C. Recklitis[1], J. Harvey[1], E. Casey[1], K. Danziger[2], J. Garber[1], L. Diller[1].* 1) Dana Farber Cancer Inst, Boston, MA; 2) Genetic Health, Inc, San Mateo, CA.

It is estimated that 1 in 900 young adults are survivors of childhood cancer. Childhood cancer survivors have unique long-term medical and psychosocial needs. In the Dana-Farber Cancer Institutes Long-term Follow-up Clinic (LTC), cancer survivors have the opportunity of meeting with a multidisciplinary team of providers. Visits are tailored to meet the survivors needs. From March 1999 to March 2000, 172 survivors were seen in the LTC; 111 new visits and 61 follow-up visits. Since the LTC opened, a total of 39 survivors requested and received genetic counseling. In these 39 cases, the underlying cancer diagnosis in the survivor was retinoblastoma (38%), leukemia or lymphoma (27%), sarcoma (13%), brain (10%), germ cell (7%), and liver/kidney (5%). Genetic counseling issues centered on concerns about second cancers and risks of cancer in offspring/siblings. Survivors had a current mean age of 15.5 (range:1-43) and were an average of 11 years from diagnosis. 19/39 (49%) had family histories suggestive of a hereditary cancer syndrome and 10/39 (26%) have undergone DNA testing. Only 2/39 (5%) had previously had genetic counseling. The genetic counseling client was the survivor in 36% of cases and the parents in 64% of cases. Counseling dilemmas have included requests for predictive genetic testing in unaffected minors and secrecy issues around the cancer diagnosis or familial genetic test result. In summary, clinics that provide services for childhood cancer survivors may be an appropriate venue for identifying families with hereditary cancer syndromes and providing reassurance to those at low risk.

Program Nr: 119 from the 2000 ASHG Annual Meeting

Can an interactive computer program educate patients about breast cancer susceptibility, and does it influence their intent to undergo testing? *M.J. Green[1], B.B. Biesecker[2], A. McInerney[2], N. Fost[3]*. 1) Department of Humanities, Penn State College of Medicine, Hershey, PA; 2) Medical Genetics Branch, NHGRI, NIH, Bethesda, MD; 3) Department of Pediatrics, University of Wisconsin, Madison, WI.

Purpose: To compare a computer-based education program with education and counseling by a genetic counselor, for their effects on knowledge of breast cancer genetics and intent to undergo genetic testing.

Methods: Women considering genetic testing for breast cancer susceptibility who had a first-degree relative with breast cancer were randomized to undergo education by genetic counselor or by interactive computer. Twenty-nine received individualized counseling about breast cancer risk and genetic testing from a genetic counselor, and 29 received education from an interactive computer program, followed by individualized counseling. Knowledge about breast cancer genetics was measured at baseline for 14 controls, and after individualized counseling or computer use for experimental groups. Intent to undergo testing was measured before and after interventions.

Results: Controls correctly answered 74.2% of knowledge questions, while those educated by a genetic counselor correctly answered 91.8%, and those educated by computer, 95.5%. This confirms our hypothesis that both counselor and computer-based interventions can significantly improve knowledge over baseline ($p<.0001$). Further, knowledge scores were significantly higher in the computer group than the counselor group ($p=.047$). Prior to either educational intervention, a majority of participants in both groups (69%) indicated they were likely (definitely + most likely) to undergo testing if a genetic test were offered to them that day. Following either educational intervention, only 44% indicated that they were likely to do so. That is, participants were 2.9 times more likely (95% CI 1.7-4.9) to say they would be tested before education than after ($p=.0002$).

Conclusions: A computer program can successfully educate patients about breast cancer susceptibility, and can influence patients' intentions to undergo genetic testing.

Program Nr: 120 from the 2000 ASHG Annual Meeting

Identifying susceptibility genes for Psoriasis and Migraine by SNP LD mapping. *R.A. Gibson, C-F. Xu, N.J. White, L. McCarthy, D. Hewett, I. Purvis, A. Roses.* UK Discovery Genetics,Glaxo Wellcome R & D, England.

Whole-genome association studies using high-density SNP markers have recently been proposed to identify susceptibility genes for common diseases. One of the key determining factors for the success and feasibility of such studies are the spacing of the SNP markers. All the markers used will have to be in LD with their nearest neighbours to enable the detection of the disease allele embedded within. The density required for such studies has been predicted by population simulation studies. However, experimental data showing the degree of the LD across long chromosome regions are sparse. In this study, we showed that common disease susceptibility genes could be isolated by SNP LD mapping. Two disease susceptibility loci, on chromosome 3 (1 Mb) and 19 (0.5 Mb) for Psoriasis and Migraine respectively were identified by linkage analysis. These loci were physically mapped using BACs, PACs and YACs. Twenty-eight and 22 SNPs were genotyped from the chromosome 3 and 19 regions respectively. The D' program was used to compare LD between markers in these regions. Our results indicated that 70% of neighbouring SNP marker pairs 2-100 kb apart showed significant LD (D>0.3). Significant association between SNP markers and disease phenotypes was also observed at both loci. Our study provided experimental evidence supporting the strategy of isolating common disease susceptibility genes in genome-wide association studies using high-density SNP markers.

Program Nr: 121 from the 2000 ASHG Annual Meeting

Large-scale SNP (single nucleotide polymorphism) discovery project in Japan. *T. Tanaka, Y. Ohnishi, R. Yamada, Y. Nakamura.* Hum Genome Cntr, Inst Med Sci, Univ Tokyo, Tokyo, Japan.

SNP is the simplest and the most frequent form of DNA polymorphism. It is thought to be widely distributed in the genome, estimated one in 1,000 bases. Because of these characteristics, SNP is expected to be of great use in the association studies on common diseases or drug sensitivities. As a first step toward association studies, we have started a large scale SNP discovery project in the Japanese population as a part of the national millennium project in Japan. Our goal is to identify 150,000 SNPs in two years. For this purpose we introduced 60 PCR machines, 12 automated liquid-handling machines, and 22 capillary-based automated sequencers.

Similar project is also in progress by the SNP consortium in the US. Although their start was one year ahead of us, our strategy is different from theirs, which we think is our advantage. Since SNPs located within the genes have more possibility to directly affect gene functions, amount of expression, and so on, we decided to concentrate on these SNPs. Our strategy is as follows; human genomic sequences are retrieved from GenBank database and PCR primers are synthesized to amplify exons, promoter regions and introns. Genomic DNA from three independent individuals are mixed into one and used as templates for direct sequencing of PCR products. In total, 24 individuals are examined in eight sample tubes. Computer program Polyphred is used to assist verification of SNPs by eye inspection, followed by construction of SNP database open to public as soon as possible. Theoretically, this system can detect SNP whose allelic frequency is 5% with the possibility of 90%. The first two-month result including the training of 30 lab members was that approximately 10 Mb have been sequenced. So far 3426 SNPs were found after inspection of about 4 Mb for verification. We are now identifying nearly 1,600 SNPs per week. SNP database obtained from our study will surely be the infra-structure for identification of genes related to common diseases or drug sensitivity and eventually enable us to make personalized medicine come true.

Program Nr: 122 from the 2000 ASHG Annual Meeting

Use of experimentally constructed haplotypes in linkage and linkage disequilibrium studies. *J.A. Douglas[1], S.B. Gruber[2], E. Gillanders[3], J.M. Trent[3], M. Boehnke[1]*. 1) Depts of Biostatistics; 2) Epidemiology, and Internal Medicine, Univ Michigan, Ann Arbor; 3) NHGRI, Bethesda, MD.

Constructing haplotypes on the basis of conventional genotype and pedigree data remains an imprecise science. We describe a novel application of a chromosome separation technology called conversion that permits unambiguous haplotype determination. Conversion takes advantage of selective retention of a subset of human chromosomes within somatic cell hybrids, isolating single copies of all desired human chromosome pairs. Identification of which homolog is retained in a particular hybrid is determined by conventional genotyping of a few markers per chromosome. Haplotypes may then be determined by genotyping each haploid hybrid. We are carrying out a full genome scan on DNA from 100 hybrids to examine chromosomal retention patterns, test the feasibility of conversion for whole genome analysis, and evaluate assumptions regarding appropriate experimental design.

We have investigated the theoretical efficiency of using haplotypes compared to conventional genotypes in linkage and linkage disequilibrium studies. In the linkage disequilibrium setting, we determined the Fisher information (with respect to haplotype frequency) provided by unrelated individuals. For two-SNP haplotypes, conversion provides 5-45% more information per subject than standard genotyping, depending on true haplotype frequencies; for five-SNP haplotypes, improvement ranges 20-92%. Since Fisher information is inversely related to sample size, conversion requires up to 12.5 times fewer subjects than standard genotyping to obtain the same information. We currently are completing similar calculations in the linkage analysis setting.

The extra cost associated with conversion includes hybrid construction and characterization, and duplicate genotyping. However, the increased information per subject reduces overall recruitment and phenotyping costs, which tend to dominate those for genotyping. The efficiency of using conversion to construct haplotypes is likely to increase even further as automated methods of genotyping continue to improve.

Program Nr: 123 from the 2000 ASHG Annual Meeting

A new method for mapping genes based on haplotype sharing. *I. Nolte, G. Spijker, G. te Meerman.* Medical Genetics, Univ Groningen, Groningen, Netherlands.

It has been shown empirically that association between genetic markers and disease alleles may be insufficient to achieve sufficient accuracy of fine mapping. E.g. in the HLA region strong association exists, but finding the causal proves to be very difficult. Systematic haplotype comparison offers an improved perspective on genetic fine-mapping. We measure the mean length of haplotype sharing both in patients and in controls. Our hypothesis is that the largest difference in length of haplotype sharing of patients and controls is found at the marker interval containing the risk allele. The physical model underlying this haplotype sharing is haplotype decay starting from the position of original mutant alleles. Our method is non-parametric and fast, which makes simulation studies possible. Time consuming methods that postulate ancestral haplotypes (Service, McPeek and MacLean) or that try to reconstruct the evolutionary tree (Lam) do not lend themselves to extensive simulation studies and are therefore difficult to validate. We studied our method with regard to power and accuracy of mapping and compared it with association and TDT. A population was simulated over 500 generations with 1000 founders with increasing exponential growth. Four disease models were used varying the genetic effect (strong or weak) and the generation of introduction of the mutants (founder or recent mutations). Our method performs best for recently introduced disease mutations. In that case both power and accuracy are higher than for association and TDT. Furthermore data simulated under the null hypothesis showed that the results of our haplotype sharing method are independent of association and TDT results. We think that these results are scalable up to 5 kb resolution for a founder population of 5000 generations old, because at that level of resolution there is still no lack of polymorphic information. Large samples will however be needed to distinguish a causal from a non-causal polymorphism. As an example of real data the hemochromatosis data of Ajioka and Thomas were analyzed. For both data sets our method identifies the locus closest to the HFE gene whereas in the original analyses the association peak was located about 1 Mb from the true locus.

Program Nr: 124 from the 2000 ASHG Annual Meeting

Human diallelic insertion/deletion polymorphisms. *N. Ghebranious[1], J. Che[1], D. David[1], Y. Fan[1], C. Zhao[1], G. Marth[2], M. Doktycz[3], J.L. Weber[1].* 1) Ctr Medical Genetics, Marshfield Medical Res Fndn, Marshfield, WI; 2) National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; 3) Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN.

Roughly 900 human diallelic insertion/deletion (indel) polymorphisms have been identified to date at Marshfield using overlapping genomic sequences and UniGene EST clusters. Overlapping BAC sequences have been the richest ore for indel mining. The great majority of the indel polymorphisms have length differences between alleles of 2 to 6 nucleotides. Allele frequencies for the less abundant alleles were about evenly distributed between 5 and 50%. Estimates of allele frequencies were obtained for four populations: Europeans, Pygmies, Japanese and Amerindians. Evolution of the diallelic indels appears similar to base substitutions. Virtually all of the human diallelic indels were monomorphic in chimps and gorillas. Deletions predominated over insertions by a ratio of about 4:1. In 70% of cases, the new allele that arose since divergence of the human/ape ancestors was the less frequent allele. A searchable database of our indels is available from the Marshfield website (www.marshmed.org/genetics). To analyze the diallelic indels, we have developed an efficient closed-tube system which utilizes allele-specific PCR primers. Successful PCR is detected by flourescence from a universal Intergen molecular beacon reagent. Initial experiments involved a single Fam reagent in two separate reactions. More recently, both alleles have been analyzed in a single reaction using Fam and Joe reagents. The assay is carried out in 384 well microtiter plates using reaction volumes as low as 500 nl. We have generated allele specific primers for 177 markers to date with > 80% success rate. We estimate that genotyping costs of diallelic polymorphisms using this closed tube system will be on the order of $0.15/genotype. This compares to a current total cost for STRPs of $0.40/genotype. Additional miniaturization of the closed-tube assay has the potential to substantially lower costs. This assay can also be applied to at least some base-substitution polymorphisms.

Program Nr: 125 from the 2000 ASHG Annual Meeting

Array-Based Candidate Gene Analysis Identify COL11A2 on 6p21.3 as Predisposing Locus for Distal Interphalangeal Joint Osteoarthritis. *J.K. Leppavuori[1], T. Pastinen[1], U. Kujala[2], J. Kinnunen[3], K. Tallroth[4], L. Peltonen[1].* 1) Dept. of Hum. Mol. Gen., Natl. Public Health Inst., Finland; 2) Unit for Sports and Exercise Med., Univ. of Helsinki, Finland; 3) Dept. of Radiology, Univ. of Helsinki, Finland; 4) ORTON Orthopaedic Hospital, Helsinki, Finland.

We have developed a high-throughput array-based genotyping system for the following candidate genes of osteoarthritis (OA):

GENE	LOCUS	GENE	LOCUS	GENE	LOCUS
AGC1	15q26	COL3A1	2q31-q32.3	COL11A2	6p21.3
PI	14q32.1	COL9A1	6q12-q13	IL1A	2q13
CRTL1	5q13-q14	COL9A2	1p33-p32.3	IL1B	2q13-q2
COMP	19p13.1	COL9A3	20q13.3	TNFA	6p21.3
COL1A1	17q21.3-q22	COL5A2	2q32-q33	TNFB	6p21.3
COL1A2	7q21.3-q22	COL10A1	6q21-q22	VDR	12q12-q14
COL2A1	12q12-13.2	COL11A1	1p21		

This setting should facilitate the analyses of not only individual candidate genes but also evaluation of the contribution of multiple genes to various forms of osteoarthritis. So far we have identified a strong association in the DIP OA study sample to three intragenic SNP:s in the COL11A2 locus. The p-value in Dismult analysis was 0.00001.

Program Nr: 126 from the 2000 ASHG Annual Meeting

Mistyping probabilities from a multipoint analysis of general pedigrees. *E. Sobel[1,3], K. Lange[1,2]*. 1) Department of Human Genetics and; 2) Department of Biomathematics, UCLA, Los Angeles, CA; 3) Centre National de Génotypage, France.

Marker genotyping errors are the skeleton in the closet of statistical genetics. Everyone knows that there is considerable mistyping in most genotype data and that even error rates of 1% to 2% can distort linkage conclusions. However, statistical analysis is usually performed assuming all the genetic data is correct. It is rare for a statistical package to either detect all likely errors or incorporate an error model that allows for them in the analysis.

We present an algorithm that provides a posterior probability of mistyping at each genotype in a pedigree. The algorithm allows for an exact analysis on small pedigrees and an approximate analysis on all pedigrees. All Mendelian errors are found. However, some genotyping errors are consistent with Mendelian inheritance. These errors are often revealed by the double recombinations they force. Through a multipoint analysis that uses all the available data, our algorithm constructs an overall probability of mistyping.

Construction of the posterior mistyping probabilities is based on the marker map and a prior error model. The marker map defines the likelihood of any recombinations inferred from the data. The error model defines the penetrance function at the marker loci, i.e., Pr(observed genotype | true genotype). The algorithm easily accommodates alternative error models. The simplest error model uses a single error rate for all mistypings. However, false homozygosity is often the most common genotyping error. We have designed an error model that incorporates this information and recognizes that misreading one allele is more common than misreading two, although there may be correlated errors as well.

This mistyping analysis has been implemented in Mendel version 4, where the analysis is exact but limited to smaller pedigrees, and in SimWalk2 version 2.7, where the analysis is an estimation but can run on large pedigrees. On both simulated and real data, these implementations demonstrate their ability to highlight genotypes that have been mistyped, even those errors consistent with Mendelian inheritance.

Program Nr: 127 from the 2000 ASHG Annual Meeting

Incidence of metabolic disorders detected by newborn screening in North Carolina using tandem mass spectrometry. *J. Muenzer[1], D.M. Frazier[1], S.D. Weavil[2], D.S. Millington[3], E.G. Moore[2], S.W. Burton[2], C.A. Currie[2], S.H. Chaing[2]*. 1) University of North Carolina, Chapel Hill, NC; 2) Division of Public Health, Raleigh, NC; 3) Duke University, Durham, NC.

The North Carolina Newborn Screening Program (NCNSP) has been screening all newborn infants using tandem mass spectrometry (TMS) for selected amino acid disorders, organic acidurias and fatty acid oxidation disorders since August 1997. Initially a statewide pilot study was conducted with Neo Gen Screening, Inc., Pittsburgh, PA, performing the TMS analysis. Based on the outcome of the pilot study, the NCNSP began performing the TMS analysis as part of the total NC newborn screening panel in April, 1999. During the 20 month pilot study with Neo Gen, 195,255 newborns were screened and 259 samples (0.13%) were identified as abnormal. A metabolic disorder was confirmed in 33 infants; 14 with medium chain acyl-CoA dehydrogenase (MCAD) deficiency, 1 long chain fatty acid oxidation disorder, 6 hyperphenylalaninemia, 1 hypermethionemia, 3 citrullinemia, 1 argininosuccinic aciduria, 5 organic aciduria and 2 classical galactosemia. The NCNSP has screened 131,776 newborns from 4-21-99 to 6-01-00. Initial cutoffs resulted in false positive detection rates of >1.9%, but revised cutoffs have resulted in a <0.85% false positive rate. A metabolic disorder was confirmed in 27 infants; 10 with MCAD deficiency, 1 SCAD deficiency, 9 hyperphenylalaninemia and 7 organic aciduria. Based on the combined data sets, about 1 in 5500 infants screened by TMS has been confirmed to have a metabolic disorder. Since the onset of TMS screening in North Carolina, 3 infants were missed by TMS screening and were diagnosed clinically before one year of age (1 with late onset methylmalonic acidemia and 2 with glutaric acidemia type I) . Newborn screening by TMS is a successful method to detect selected metabolic disorders, but false negative results have occurred. Close coordination between the TMS screening laboratory and the metabolic clinic/biochemical geneticists is needed to determine screening parameters, adjust the cutoff levels to reduce false positive and false negative results and facilitate clinical follow-up.

Program Nr: 128 from the 2000 ASHG Annual Meeting

Outcome of First Two Years of Neonatal Screening for Glutaric Acidemia Type 1 in Oji-Cree. *C.R. Greenberg[1], R. Thompson[2], S. Busca-Owczar[1], F.A. Booth[1], A.N. Prasad[1], L.E. Seargeant[1], L.A Dilling[1], C. Prasad[1], P. Wood-Steinman[3], B. Martin[1]*. 1) University of Manitoba, Winnipeg, Canada; 2) Cadham Provincial Laboratory, Winnipeg, Canada; 3) Island Lake Tribal Council, Winnipeg, Canada.

Glutaric acidemia type 1 (GA1) is an autosomal recessive inborn error of metabolism common in a Canadian Aboriginal population of Oji-Cree ancestry in NE Manitoba and NW Ontario. A single homozygous mutation in *GCDH* has been associated with the Oji-Cree form of GA1 (Greenberg et al, 1995). Evidence is accumulating that presymptomatic detection and treatment may prevent the acute encephalopathy and irreversible brain damage characteristic of this disorder. We thus embarked on a DNA-based neonatal screening program in our high-risk population with the aim of improving clinical outcome. In the first 2 years of the program, 707 neonates have been screened, 3 affected babies have been diagnosed and 44 carriers have been identified.A low protein diet, carnitine, riboflavin and aggressive treatment of intercurrent illnesses were instituted in the 3 affected babies identified by newborn screening. Treatment of the first identified affected baby was attempted in her home community but her clinical outcome has not been satisfactory. The families of the next 2 affected babies were relocated to Winnipeg to ensure rapid hospitalization and aggressive treatment of intercurrent illnesses. Brain CT scans of these 2 asymptomatic babies, at 3 and 4 months of age respectively, showed mild fronto-temporal atrophy, widening of the Sylvian fissures and normal basal ganglia. Subsequently, 1 of the latter 2 babies, at 5 months of age presented with acute dystonic encephalopathy of unknown etiology and in spite of rapid institution of treatment, significantly abnormal neurological findings are now apparent. We have acknowledged the social implications of early family relocation to Winnipeg but the high-risk Oji-Cree population and its community leadership has sanctioned GA1 neonatal screening. Long-term evaluation of our program is necessary to determine whether improved clinical outcome can be achieved by neonatal screening for the Manitoba variant of GA1.

Program Nr: 129 from the 2000 ASHG Annual Meeting

A population-based study of the effect of the HFE C282Y and H63D mutations on iron metabolism. *O.T. Njajou, R. Osborne, N. Vaessen, J. Vergeer, A. Hofman, C.M. Van Duijn.* Epidemiology and Biostatistics, Erasmus University Rotterdam, Rotterdam, Rotterdam, The Netherlands.

Hemochromatosis is a common recessive disorder in populations of Caucasian origin. The disease is characterised by iron accumulation throughout the body. Iron accumulation is associated with a wide spectrum of disorders including diabetes, cardiovascular diseases and stroke. There is increasing interest in the effect of mutations in the hemochromatosis gene (HFE) on iron metabolism, in particular in those heterozygous for the common mutations (C282Y and H63D). We studied the relationship between these HFE mutations and iron, ferritin, transferrin and transferrin saturation in 2300 subjects aged 55-75 years drawn from a population-based study, the Rotterdam study of whom 900 were randomly selected. In this random population, one subject (0.3%) was homozygous and 37 (10.3%) heterozygous for the C282Y mutation in men and 3 (0.6%) were homozygous and 79 (14.9%) heterozygous in women. For the H63D mutation, 17 (4.7%) were homozygous and 76 (21.1%) heterozygous in men and 16 (3.0%) were homozygous and 130 (24.4%) heterozygous in women. Iron metabolism was studied in a subgroup of individuals stratified by genotype and sex (n=237). In men, we found higher levels of iron, ferritin and transferrin saturation in those hetero-or homozygous for the C282Y mutation compared to those homozygous for the wildtype allele. Also men heterozygous or homozygous for the H63D mutation showed higher levels of iron, ferritin and transferrin saturation. In women, homozygosity of the C282Y or H63D mutation and compound heterozygosity was found to effect iron metabolism. Our study shows that the H63D mutation has an effect on iron metabolism. Our data suggest that heterozygosity and homozygosity for the C282Y or the H63D mutation is associated with a disturbed iron metabolism in men. In females, an effect could be shown only for those homozygous or compound heterozygous, suggesting that women may be protected from HFE related pathology up until menopause through menstruation.

Program Nr: 130 from the 2000 ASHG Annual Meeting

Partial deficiency of phosphomannomutase: a pitfall in the diagnosis of congenital disorders of glycosylation (CDG-Ia). *G. Matthijs[1], S. Grünewald[1], E. Schollen[1], G. Berghenouse[3], J. Jaeken[2], E. Van Schaftingen[3].* 1) Center for Human Genetics, University of Leuven, Leuven, Belgium; 2) Center for Metabolic Diseases, University Hospital, Leuven, Belgium; 3) Laboratory of Physiological Chemistry, ICP, University of Louvain, Brussels, Belgium.

The most frequent type of congenital disorders of glycosylation (CDG) is due to mutations in the phosphomannomutase 2 (PMM2) gene. Up to now, we have identified more than 30 different mutations in the PMM2 gene in 100 CDG-Ia families. Routinely, PMM activity is measured in fibroblasts of patients with a type I pattern on isoelectric focusing of serumtransferrin (IEF). The majority of CDG-Ia patients have PMM activities below 10% of normal. However, in some CDG patients, we measured residual PMM activities that reached heterozygous values. This observation urged us to re-evaluate all patients with the typical IEF pattern and a clinical presentation suggestive of (a milder form) of classical CDG-Ia. We now have documented 28 patients - in a series of over one hundred - with a partial deficiency of PMM (over 25% of normal) and identified PMM2 mutations in 24 of them. The highest PMM value (3.29 mU/mg protein) was found in a patient, compound heterozygous for the I120T and V231M mutations. Preliminary data show that in leukocytes of patients with partial deficiency in PMM, the residual activity is consistently lower than in fibroblasts (below 7 % of the control). Thus, the PMM assay in leukocytes appears to be a more reliable test for CDG-Ia. In conclusion, patients with high residual activity of PMM in fibroblasts might still harbour mutations in PMM2, and further scrutiny is warranted, especially if the clinical picture strongly suggests CDG-Ia.

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Program Nr: 131 from the 2000 ASHG Annual Meeting

Desmosterolosis Presenting With Multiple Congenital Anomalies And Profound Developmental Delay. *H.C. Andersson[1], L.C. Kratz[2], R.I. Kelley[2]*. 1) Human Gen Prog/Hayward Gen Ctr, Tulane Univ Medical Ctr, New Orleans, LA; 2) Kennedy Krieger Institute, Baltimore,MD.

Desmosterol is a 27-carbon sterol intermediate in the cholesterol biosynthetic pathway. No living patient with deficiency of desmosterol conversion to cholesterol has ever been described, although Fitzpatrick et al reported a stillborn fetus with tissue elevations of desmosterol and mildly increased plasma desmosterol levels in the parents. We describe a first-born infant born at 42 weeks gestation following an uncomplicated pregnancy with dysmorphic facial features (downslanting palpebral fissures, micrognathia), submucous cleft palate, clubfoot, profound microcephaly, agenesis of the corpus callosum, 2-vessel cord and persistent patent ductus arteriosus. Additional studies included a normal prometaphase chromosome analysis, normal renal and cardiac sonography (other than a PDA), and normal TORCH titers. Plasma sterol quantification (GC-MS) looking for a disorder of cholesterol synthesis found normal cholesterol and 7-dehydrocholesterol levels but a 100-fold increased leve of desmosterol (60mcg/ml; nl 0.5mcg/ml) suggestive of steroid-24-reductase deficiency. Additional biochemical studies in the patient and his parents were performed. Both parents had moderately elevated plasma desmosterol levels (mother: 1.4mcg/ml; father: 1.8mcg/ml), suggestive of heterozygosity for steroid-24-reductase deficiency. Analysis of sterol synthesis in transformed lymphoblasts documented deficient conversion of desmosterol to cholesterol and low cholesterol levels in the patients cells. At age 3.5y, the patient is standing, uses 5 words, and has no major medical problems. This unique patient broadens the spectrum of inborn errors of cholesterol biosynthesis and offers important contrasts to the Smith-Lemli-Opitz syndrome. Photographs and video of the patient will be presented.

Program Nr: 132 from the 2000 ASHG Annual Meeting

Retinal dysfunction in patients with the Smith-Lemli-Opitz Syndrome (SLOS). *E.R. Elias[1], A. Fulton[2], D.L. Mayer[2], R.M. Hansen[2]*. 1) Coordinated Care Svc/Fegan 10, Children's Hosp, Boston, MA; 2) Dept. of Ophthalmology, Children's Hospital, Boston, MA.

Patients with SLOS have an inborn error in the cholesterol pathway, resulting in severe cholesterol deficiency. Patients with abetalipoproteinemia, another disorder associated with low cholesterol, develop retinitis pigmentosa (RP) and visual deficits. Following the detection of severe RP and night blindness in a 16 year old SLOS patient, 7 additional SLOS patients were assessed.

Eight children (ages 2-16 years) with biochemically confirmed SLOS, and pretreatment cholesterol levels ranging from 20-124 mg/dl, underwent electroretinogram (ERG), and fundoscopic evaluation under anesthesia. Visual fields and acuities were assessed using behavioral methods.

A severe pigmentary retinopathy was demonstrated in one older patient with severe SLOS, who also had been treated longterm with Mellaril. ERG's demonstrated abnormal retinal function in all eight patients studied. Rod sensitivity was decreased to 50% or less of normal for age. Saturated rod photoresponse amplitude was less markedly affected. Deactivation of the rod response was slower than normal. Cone mediated responses were abnormal in only one patient. Acuities were below normal for age. There were no major visual field deficits.

All eight SLOS patients demonstrated photoreceptor (rod cell) dysfunction on ERG, irregardless of age or degree of metabolic defect. This is the first study to report the adverse effects of the cholesterol defect in SLOS on rod cells, which normally undergo rapid cell turnover of their lipid-laden outer segments. Serial testing of SLOS patients is planned to determine whether treatment with high dose cholesterol supplementation can improve retinal and visual function in this disorder. The use of Mellaril and other drugs known to cause RP should be used with caution in SLOS patients.

Program Nr: 133 from the 2000 ASHG Annual Meeting

Repeat extension in XLGNAS1 leads to enhanced Gs signaling and is a risk factor for bleeding and mental retardation. *K. Freson[1], M.F. Hoylaerts[1], J. Vermylen[1], C. Van Geet[1,2]*. 1) Center for Molecular and vascular biology; 2) Department of Pediatrics, University Hospital Gasthuisberg, Leuven, Belgium.

Alternatively spliced GNAS1 and XLGNAS1 are located on the imprinted chromosomal region 20q13. Whereas loss-of-function mutations in GNAS1 lead to pseudohypoparathyroidism, gain-of-function mutations as in the McCune Albright syndrome are only viable when mosaic. We studied the Gs pathway in platelets by an aggregation-inhibition test in candidate patients. In some patients, platelet aggregation was strongly inhibited at lower levels of different Gs agonists in comparison to controls, suggesting a Gs hyperfunction. Basal cAMP was normal in platelets, but after Gs stimulation, a stronger than normal rise in cAMP was triggered. The individual degree of platelet aggregation inhibition correlated with the increased Gsa protein level in the platelet membranes. No mutations were found in GNAS1 but we presently report for the first time a gene defect in the maternally imprinted XLGNAS1. Two extra repeats via a 36 bp insertion and a missense mutation upstream of the insertion were detected in XLGNAS1 from 12 patients. This paternally inherited repeat extension is associated with overexpression of GNAS1, presumably by a deregulation in the alternative expression of paternal XLGNAS1 and GNAS1. These patients clinically manifest an increased bleeding tendency after challenging the hemostatic system, and a variable degree of mental retardation. The frequency of this mutation in a group of patients with unexplained mental retardation was 5,5 %. Half carry a maternally inherited repeat extension, have a weak positive to normal aggregation test and associated membrane-bound Gsa level. The mutation frequency in a control population is 2,5 %. We functionally tested two controls with a paternally inherited repeat extension and found a moderate but significantly increased sensitivity towards Gs agonists in platelets and an associated increased Gsa overexpression. A tested control with a maternally inherited mutation was negative. Studies are ongoing to determine the potential role of imprinting in the relation between the XLGNAS1 mutation, the Gsa overexpression and the variable phenotype.

Program Nr: 134 from the 2000 ASHG Annual Meeting

Enzyme replacement therapy in Fabry disease: Results of a placebo-controlled phase 3 trial. *C.M. Eng[1], P. Cochat[1], W.R. Wilcox[1], D.P. Germain[1], P. Lee[1], S. Waldek[1], L. Caplan[1], H. Heymans[1], T. Braakman[2], M.A. Fitzpatrick[2], P. Huertas[2], M.W. O'Callaghan[2], S. Richards[2], P.K. Tandon[2], R. Desnick[3]*. 1) The International Fabry Disease Study Group; 2) Genzyme Corporation, Cambridge, MA; 3) Mount Sinai School of Medicine, New York, NY.

Fabry disease (a-galactosidase (a-GAL) deficiency) results in the progressive lysosomal accumulation of the glycosphingolipid GL-3 in tissues throughout the body. Accumulation in the vascular endothelium of kidney, heart and brain is responsible for early death from failure of these organ systems. We conducted a multinational, double-blind, placebo-controlled trial in 58 patients (8 centers, 4 countries). Each patient received an infusion of recombinant human a-GAL (r-haGAL) enzyme or placebo every two weeks for 20 weeks. The patients ranged in age from 16-61. The primary efficacy endpoint was the clearance of stored GL-3 in the kidney vasculature. GL-3 storage was assessed independently by three pathologists blinded to treatment and timepoint. Twenty of 29 patients treated with r-haGAL achieved the prospectively defined efficacy endpoint. In contrast, no patient treated with placebo achieved the primary endpoint ($p<0.0001$). Accumulation of GL-3 in the vasculature of the kidney, heart and skin was also evaluated at baseline and at week 20. The amount of GL-3 decreased significantly in response to r-haGAL - in each organ and across all organs ($p<0.001$). Infusions were reasonably well tolerated. Adverse event profiles of r-haGAL-treated and placebo-treated patients were comparable with the exception of transient mild to moderate infusion-related reactions in the r-haGAL-treated patients. Twenty-four of 29 treated patients developed IgG antibodies to a-GAL. Seroconversion did not appear to influence the efficacy of r-haGAL treatment. All patients continued treatment in an open-label extension study. In conclusion, r-haGAL successfully cleared GL-3 from the vasculature of the kidney, heart and skin.

Program Nr: 135 from the 2000 ASHG Annual Meeting

Efficacy and safety of enzyme replacement therapy for Fabry disease demonstrated by a double-blind placebo-controlled trial. *R. Schiffmann[1], J.B. Kopp[2], H. Austin[2], D.F. Moore[1], S. Sabnis[3], T. Weibel[1], J.E. Balow[2], R.O. Brady[1]*. 1) Developmental and Metabolic Neurology Branch/NINDS/NIH, Bethesda, MD; 2) Metabolic Diseases Branch/NIDDK, NIH, Bethesda, MD; 3) Division of Nephropathology, AFIP, Washington DC.

Fabry disease is an X-linked recessive storage disorder caused by a deficiency of the lysosomal enzyme a-galactosidase A (AGA). Globotriaosylceramide (Gb3), the glycolipid substrate of this enzyme, accumulates within susceptible cells. Clinical manifestations include recurrent episodes of severe pain, progressive renal, cardiac and cerebrovascular deterioration with premature death. No effective treatment has existed. In the present study, 26 hemizygous male patients were randomized to receive 12 infusions of 0.2 mg/kg of AGA or placebo. The infusions were given every two weeks over 20-40 minutes. Outcome measures included neuropathic pain measured while off analgesics using the Brief Pain Inventory, kidney function and structure, cerebral blood flow, and Gb3 levels in plasma, urine and kidney. Using intent-to-treat analysis, pain-at-its-worst scores on a 0-10 scale decreased from 6.21 to 4.29 (AGA) 7.25 to 6.83 (placebo). The difference between the groups was significant ($p=0.021$). Severity scores, pain-related quality of life scores, and mean number of days off pain medications also decreased significantly in the treated group compared to controls. Mean creatinine clearance increased by 4 ml/min in patients receiving AGA while it decreased by 19 ml/min in those on placebo ($p=0.016$). Mean inulin clearance decreased by 6 ml/min in the AGA group and by 20 ml/min on placebo ($p=0.17$). In AGA-treated patients, there was an increase in the fraction of renal glomeruli with normal mesangial architecture ($p<0.01$). Renal cellular inclusions decreased with treatment ($p=0.07$). Blood flow to vulnerable brain areas significantly increased with AGA. Decreased Gb3 levels mirrored the clinical improvements. AGA was well tolerated. We conclude that enzyme replacement therapy with AGA is safe, reduces neuropathic pain, stabilizes renal function and improves glomerular mesangial histology and cerebral perfusion in Fabry disease.

Program Nr: 136 from the 2000 ASHG Annual Meeting

Enzyme supplementation for treatment of arthero-/arterio-sclerosis using lysosomal acid lipase (LAL). *G.A. Grabowski[1], D. Witte[1], M. Levine[2], S.C. Schiavi[2], H. Du[1]*. 1) Div Human Genetics, Childrens Hosp Medical Ctr, Cincinnati, OH; 2) Genzyme Corporation, Cambridge, MA.

Athero/arterio- sclerosis is a major cause of mortality and morbidity in westernized nations and a leading cause of death in the United States. Several surgical, pharmacologic, and life-style interventions have incrementally improved the predisposition to or recurrence of myocardial infarctions. We present here "proof of principle" of targeted enzyme supplementation for decreasing the progressive coronary wall lipid deposition in a mouse model of athero/arterio-sclerosis. Mice with low density lipoprotein receptor deficiency (LDLR-KO) develop predictable coronary atheromata after receiving a high cholesterol/high fat diet (HC/HF). These mice have normal levels of LAL in their tissues. After 2.5 mo. on HC/HF, LDLR-KO mice were bolus injected intravenously, every third day for 10 doses (~1.5 U/dose), with purified mannosyl-terminated human LAL produced in Pichia pastoris (phLAL). HC/HF was continued throughout the study period. A cohort of age matched LDLR-KO mice on HC/HF were injected with saline on the same schedule. The enzyme is recognized by mannose receptors expressed on J774E cells and is taken up by macrophages in vivo. Tissues were harvested after the final injections. As expected liver and splenic histology was the same in both groups. Biochemical analyses of liver, spleen and small intestine showed minor differences in total cholesterol, and ~35-50% reductions in TG levels in the treated vs untreated cohorts. Both cohorts remained hypercholesterolemic. Saline injected mice showed extensive infiltration of the coronary ostia and aortic root with foamy macrophages filled with cholesterol crystals. Mice receiving the phLAL had essentially no foamy macrophages or cholesterol deposition in the same regions, nor was there evidence for other coronary artery pathology. The infusions were well tolerated with no observable adverse effects on the treated mice during the study period. These results indicate the potential utility of such enzyme supplementation for the treatment of this major health problem.

Program Nr: 137 from the 2000 ASHG Annual Meeting

Suppression of polyglutamine toxicity by a *Drosophila* homologue of myeloid leukemia factor 1. *P. Kazemi-Esfarjani, S. Benzer*. Division of Biology, California Inst of Technology, Pasadena, CA.

In Huntington's disease (HD) and several other hereditary neurodegenerative disorders, abnormally long polyglutamine tracts within specific proteins are the major contributing factors to selective neuronal toxicity. We developed a *Drosophila*model for HD to screen for the genetic factors that modify the polyglutamine toxicity associated with the disease. The expression of peptides containing an expanded polyglutamine (127 glutamines or 127Q) and a hemagglutinin (HA) protein tag in the eye resulted in collapse of the eye and loss of pigmentation. In sections, anti-HA antibody revealed abundant inclusions typically found in HD brain tissue. Genetic screening was carried out by crossing127Q fly with 7000 other fly lines, each harboring a randomly-inserted, genomic P-element that drives the expression of flanking downstream DNA. The resulting F1 progeny were screened for changes in eye morphology and pigmentation. One such P-element insertion, EU2490, produced a moderate improvement. To identify the gene downstream of EU2490, the genomic DNA fragment flanking it was cloned and sequenced. It matched a cDNA in the Berkeley Drosophila Genome Project database. This cDNA encodes a predicted protein of 273 amino acids with a molecular weight of 30 kD and is 32% identical and 49% similar to the human myeloid leukemia factor 1 (MLF1), involved in the chromosomal translocation t(3:5)(q25.1,q34) associated with myelodysplastic syndrome and acute myeloid leukemia. We, therefore, refer to it as *Drosophila*myeloid leukemia factor (dMLF). To confirm that this gene is responsible for the improved eye phenotype, and not a gene downstream of it, fly lines transgenic for *dmlf*cDNA plasmid were established. Similar to EU2490 P-element insertion, overexpression of *dmlf*cDNA from a single transgene partially suppressed the eye phenotype caused by 127Q. Combined expression of two *dmlf*transgenes resulted in a more dramatic improvement. Nevertheless, numerous polyglutamine aggregates were still present in the retina. Therefore, dMLF appears to improve cell survival without affecting the accumulation of polyglutamine peptides or their aggregates.

Program Nr: 138 from the 2000 ASHG Annual Meeting

Modification of the CFTR splicing pattern by cellular and viral splicing factors in CFTR expressing cells. *M. Nissim-Rafinia, O. Chiba-Falek, B. Kerem.* Dept Genetics, Hebrew Univ, Jerusalem, Israel.

Variable levels of aberrantly spliced CFTR transcripts, carrying splicing mutations, were shown to correlate with variable CF severity. The mechanism underlying this variability was suggested to involve splicing factors known to promote exon skipping and /or inclusion. Previous studies, in CFTR non-expressing cells, have shown that overexpression of cellular and viral splicing factors can modify the splicing pattern of CFTR minigenes carrying the splicing mutation 3849+10kbC®T and the 5T allele (Nissim-Rafinia et al. HMG 2000 in press, Pagani et al. JBC 2000 in press). Here we focused on the modulation effect of the splicing factors, in epithelial cell lines expressing the CFTR gene. We studied the RNA splicing pattern expressed from minigenes and from the endogenous gene carrying CFTR splicing mutations. The splicing pattern of the 5T minigene (p5T) in 3 human epithelial cell lines, from colon (HT29), trachea (IB3) and pancreas (PANC-1) was studied. The level of the aberrantly spliced transcripts varied (13%-30%) among the cell lines. Overexpression of ASF/SF2 and hnRNP A1 promoted exon skipping and resulted in a substantial increase in the level of aberrantly spliced mRNA, in all analyzed cell lines, except for PANC-1 in which hnRNP A1 had no effect. Overexpression of the viral splicing factor, E4-ORF3, resulted in the antagonistic effect thus, promoted exon inclusion in all the cell lines. Thus, overexpression of cellular and viral splicing factors can modulate the splicing pattern of CFTR minigenes carrying splicing mutations in CFTR expressing cells. We further extended our analysis to the endogenous CFTR gene. We established an epithelial cell line from a nasal polyp of a CF patient carrying the splicing mutation 3849+10kb C®T. RT-PCR revealed that 19%±5% of the transcripts are aberrantly spliced. This cell line provides a system to study the effect of the splicing factors on the endogenous splicing pattern of the 3849+10kb C®T mutation, the results of which will be discussed. In summary, this study provides a significant step towards understanding the regulation of alternative splicing pattern of CFTR alleles carrying splicing mutations.

Program Nr: 139 from the 2000 ASHG Annual Meeting

The Synthesis, Characterization, and *in vivo* Study of two Tetrahedral Phosphinate Transition-state-mimic Inhibitors of Fumarylacetoacetase: Possible Utility for Liver Repopulation Protocols. *R.L. Bateman[1,2], D.E. Timm[3], J. Witte[2], K. Manning[1], M. Grompe[1], R.W. McClard[2].* 1) Dept. of Molecular and Medical Genetics, Oregon Health Sciences Univ., Portland, OR; 2) Dept. of Chemistry, Reed College, Portland, OR; 3) Dept. of Biochemistry, Indiana Univ., Indianapolis, IN.

Fumarylacetoacetate hydrolase (FAH, E.C. 3.7.1.2) catalyzes the final step of tyrosine catabolism to produce fumarate and acetoacetate. Hereditary tyrosinemia type I (HT I) results from the lack of this enzyme. A murine model for the disease has been produced, and a selective growth advantage for transplanted wild type hepatocytes has been demonstrated. Thus, a pharmacologic method for enzyme inactivation may be utilized for liver repopulation protocols.

In order to further characterize the mechanism of FAH catalysis and address the need for FAH inhibitors, two phosphinate transition-state-mimic inhibitors were produced. HMPOBA [4-(Hydroxymethylphosphinoyl)-3-oxo - butanoic acid, K_i = 37.1 ± 7.6 mM, K_m = 0.51 ± 0.11 mM for recombinant human enzyme] was complexed with the mouse enzyme, and the crystal structure resolved to 1.3 Å. The compound was designed with a tetrahedral phosphinoyl moiety in place of the substrate's carbonyl adjacent to the scissle linkage. Another compound that more closely mimics the proposed transition state (TS) intermediate, CEHPOBA (4-[(2-Carboxyethyl)-hydroxyphosphinoyl]-3-oxo-butanoic acid) was also produced. This compound mimics the analogus TS intermediate for the hydrolysis of succinylacetoacetate by FAH, and inhibition assays demonstrate strong inhibition with slow onset (K_is not yet ascertained).

In vivo studies with CEHPOBA demonstrated increased levels of succinylacetone (a metabolite seen in patients with HT I) in serum after repeated injections (0.5-2.0 mmol/g, daily, I.P.) in mice. Also, following a single injection (2 mmol/g, I.P.), mouse liver homogenates had no measurable FAH activity. Use of such compounds in repopulation protocols may allow *in vivo* selection of donor hepatocytes in mice.

Program Nr: 140 from the 2000 ASHG Annual Meeting

Pharmacologic Rescue of Lethal Seizures in a Murine Knockout Model of Succinic Semialdehyde Dehydrogenase (SSADH) Deficiency. *M.J. Taylor[1], B.M. Hogema[1,2], C. Jakobs[2], R.B.H. Schutgen[2], W. Froestl[3], O.C. Snead[4], M. Grompe[1], K.M. Gibson[1]*. 1) Molec and Med Genet, Oregon Health Sci Univ, Portland, OR; 2) Metab Unit, Dept of Clin Chem, Free Univ Hosp, Amsterdam; 3) Novartis, Basel, Switzerland; 4) Dept of Neurol, Hosp for Sick Children, Toronto, Canada.

SSADH deficiency is a rare defect of GABA degradation associated with 4-hydroxybutyric (GHB) aciduria in affected patients. To explore pathomechanisms and develop preclinical treatment paradigms, we developed a targeted murine knockout of SSADH deficiency in C57/129 mice. In the first two weeks of life, mutant (-/-) mice showed decreased body weight. At postnatal day 17-20, repetitive seizures began, with rapid death in all -/- animals. SSADH activities in mouse tissue homogenates (nmol/min/mg protein) were: +/+ brain (n=34, 16.5 ± 1.3 (SEM)), and liver (n=36, 14.4 ± 1.1); +/- brain (n=11, 8.5 ± 0.7), and liver (n=14, 6.9 ± 0.6), indicating a gene-dose effect. Affected brain (n=15) and liver (n=19) had nil SSADH activity. Urine GHB and total GABA (umol/L) from +/+ and +/- mice were indistinguishable (GHB, n=12, 5.5-19.1; total GABA, n=5, 111-195), but were increased in two -/- mice (GHB, 1024 and 1350; total GABA, 446 and 707). GHB and total GABA in +/+ and +/- mice tissue extracts overlapped (brain GHB, 0.07-0.22 (umol/g protein), n=11, and liver GHB, 0.02-0.19, n=12; brain total GABA 44-71, n=4, and liver total GABA 0.8-1.7, n=6) but were elevated in -/- mice (brain GHB (n=2), 5.3 and 5.4, and liver GHB (n=5), 0.9-2.5; brain total GABA (n=2) 204 and 218, and liver total GABA (n=3) 4.1-4.3). To test if GHB in -/- mice acted on the $GABA_B$ receptor, CGP 35348 ($GABA_B$ receptor antagonist) and taurine(2-aminoethanesulfonic acid) were administered orally to -/- mice at 100 mg/kg/day. CGP 35348 led to 90.9% survival past day 62 (currently up to 111 days); taurine administration led to 66.7% survival beyond 27 days (currently up to 46 days). These data indicate that GHB acts at the $GABA_B$ receptor in -/- mice. The knockout model is useful to assess: 1) preclinical treatment strategies and pathomechanisms associated with human SSADH deficiency; and 2) pre- and postnatal pathophysiologic effects of chronic GHB exposure.

Program Nr: 141 from the 2000 ASHG Annual Meeting

A NOVEL DYNAMIN-RELATED PROTEIN IS MUTATED IN DOMINANT OPTIC ATROPHY. *C. Delettre[1], G. Lenaers[2], J.M. Griffoin[1], N. Gigarel[3], C. Lorenzo[2], P. Belenguer[2], L. Pelloquin[2], J. Grosgeorge[4], C. Turc-Carel[4], C. Astarie-Dequeker[2], B. Arnaud[5], B. Ducommun[2], J. Kaplan[3], C.P. Hamel[1,5]*. 1) Inserm U. 254,Laboratoire de Neurobiologie de l'audition, Montpellier, France; 2) Laboratoire de Biologie Cellulaire et Moléculaire du Contrôle de la Prolifération, Université Paul Sabatier, Toulouse, France; 3) Inserm U. 393, Handicaps génétiques de l'enfant, Hôpital Necker-Enfants malades, Paris, France; 4) CNRS/UNSA UMR 6549, Faculté de Médecine, Nice; 5) Service d'Ophtalmologie, Hôpital Gui de Chauliac, Montpellier, France.

Dominant optic atrophy (DOA) is the commonest form of inherited optic atrophy with an estimated frequency of 1:50,000. This non syndromic optic neuropathy with an insidious onset of variable visual loss presents optic nerve pallor, centro-coecal visual field scotoma and colour vision deficit. Genetic linkage studies have localised one dominant optic atrophy gene, OPA1, to chromosome 3q28-q29. In this study we describe a novel nuclear gene MSP1 that maps within the OPA1 candidate region and encodes a mitochondrial dynamin-related protein, that participates to the mitochondrial network. Because of clinical and morphological similarities betwen dominant optic atrophy and mitochondrial optic neuropathy found in Leber's optic atrophy including the loss of retinal ganglion cells, we reasoned that this gene might be an excellent candidate gene for DOA. We report the genomic organisation of this gene which spans over 69 kb of genomic DNA interrupted by 29 exons. We screened the entire coding sequence for probands and relatives from 6 unrelated families with DOA and we identified 4 differents mutations including framshift and misense mutations, that segregate with the disease. In addition, we observed an abnormal distribution of mitochondria in monocytes of a patient with DOA compared to control cells. This novel dynamin-related protein mutated in dominant optic atrophy demonstrate that mitochondria play a crucial role in retinal ganglion cell pathophysiology. These finds may prove to be a great value in the understanding of other primary ganglion cell diseases.

Program Nr: 142 from the 2000 ASHG Annual Meeting

Functional analyses of lens aquaporin mutants linked to human cataracts. *P.J. Francis[1,2], J.-J. Chung[3], M. Yasui[3], V. Berry[1], M.K. Wyatt[4], G. Wistow[4], A.T. Moore[2,5], P. Agre[3], S.S. Bhattacharya[1].* 1) Molecular Genetics, Institute of Ophthalmology, London, UK; 2) Moorfields Eye Hospital, London, UK; 3) Department of Physiology, Johns Hopkins Medical School, Baltimore, MD; 4) National Eye Institute, National Institutes of Health, Bethesda, MD; 5) Addenbrookes Hospital, Cambridge, UK.

Background: We recently identified two families with phenotypically distinct dominantly inherited cataracts (a condition in which the crystalline lens of the eye becomes opacified) that carried novel point mutations in the gene encoding major intrinsic protein (also referred to as AQP0), a member of the aquaporin family of transmembrane water channels (Nature Genetics 2000; 25:15-17). To establish pathophysiological relevance to cataract formation, the Xenopus laevis oocyte expression system was employed to evaluate functional defects in the mutant proteins, E134G and T138R.

Methods: Full length MIP cDNA was cloned from a human placental library and site-directed mutagenesis used to introduce the mutations. Both wild-type and mutant proteins were expressed in Xenopus laevis oocytes (following cRNA micro-injection) and their effects on cell membrane water permeability (Pf) assessed by the oocyte swelling assay. Cellular targeting of the MIP was detected by immunofluorescence.

Results: Expression of wild-type MIP conferred a three-fold increase in oocyte Pf compared to controls. In contrast, both mutations abolished membrane water channel activity when expressed alone and significantly reduced Pf when co-expressed with wild-type MIP. Immunofluorescence indicated that these findings resulted from reduced protein expression and impaired trafficking to the plasma membrane.

Conclusions: These studies establish a causative role for MIP in human cataractogenesis and explain how the mutations may exert a dominant-negative effect.

Program Nr: 143 from the 2000 ASHG Annual Meeting

Collagen XVIII, containing an endogenous inhibitor of angiogenesis, plays a critical role in the maintenance of retinal structure and in neural tube closure (Knobloch syndrome). *A.L. Sertie[1], O. Suzuki[1], V. Sossi[2], F. Kok[1], A.E. Czeizel[3], M. Monteiro[1], J. Murray[4], A. Camargo[1], N. Fukai[5], M. Zatz[1], B.R. Olsen[5], C. Brahe[2], M.R. Passos-Bueno[1]*. 1) University of Sao Paulo, SP, Brazil; 2) Catholic University, Rome,Italy; 3) Dept. Human Genetics & Teratology, Hungary; 4) University of Iowa, Iowa, USA; 5) Harvard Medical School, Boston, USA.

Knobloch syndrome (KS) is an autosomal recessive disorder defined by the occurrence of high myopia, vitreoretinal degeneration with retinal detachment, macular abnormalities and occipital encephalocele. The KS gene had been assigned to a 4.3 cM interval at 21q22.3 by linkage analysis of a large Brazilian family. We reconstructed the haplotypes of this family with 10 additional markers (5 novel ones) and narrowed the candidate interval to a region of less than 245 kb, which contains 24 ESTs, the KIAA0958 gene and the 5' end of the *COL18A1* gene. We identified a homozygous mutation at the AG consensus acceptor splice site of *COL18A1* intron 1 in all KS patients from the above family, which was not found among 140 control chromosomes. *COL18A1* with 43 exons is transcribed in 2 isoforms: One short mRNA variant (exons 1, 2 and 4 to 43) and one long variant (exons 3 to 43). Therefore, the mutation found in intron 1 of the KS Brazilian patients may cause truncation of only the a1(XVIII) collagen short form. Three different frameshift changes predicting a premature stop codon in exons 10, 36 and 41 were found in the *COl18A1* alleles in 3 out of 5 other unrelated KS patients, thus confirming that mutations in this gene cause this syndrome. We also demonstrated, through RT-PCR and/or immunohistochemistry, that there are 3 different *COL18A1* isoforms, but only the short form is expressed in human retina. These findings demonstrate that KS is caused by mutations in *COL18A1*, which may have a major role in determining retinal structure and closure of the neural tube. It is not known, however, if the KS phenotype is due to absence of type XVIII collagen or of endostatin, an endogenous inhibitor of angiogenesis released from the C-terminus of *COL18A1*, or due to the lack of both.FAPESP,HHMI,CNPq, PRONEX.

Program Nr: 144 from the 2000 ASHG Annual Meeting

Identification of the gene responsible for dominant optic atrophy (OPA1) on chromosome 3q28. *C. Alexander[1,2], M. Votruba[1], U.E.A. Pesch[2], D.L. Thiselton[1], S. Mayer[2], A. Moore[1], M. Rodriguez[3], U. Kellner[4], B. Leo-Kottler[2], G. Auburger[5], S.S. Bhattacharya[1], B. Wissinger[2]*. 1) Dept. Molecular Genetics, Inst. of Ophthalmology, UCL, UK; 2) Molecular Genetics Lab, University Eye Hospital, Tuebingen, Germany; 3) Center of Medical Genetics, Sancti-Spiritus, Cuba; 4) Eye Department, University Clinics Benjamin Franklin, Berlin, Germany; 5) Dept. Neurology, University Hospital Duesseldorf, Germany.

Autosomal dominant optic atrophy (DOA) is the most prevalent hereditary optic neuropathy resulting in a progressive loss of visual acuity, centrocoecal scotomas, bilateral temporal atrophy of the optic nerve with an onset within the first two decades of life. Linkage studies have mapped the predominant locus (OPA1, MIM #165500) for DOA on chromosome 3q28-q29. We have established a PAC contig covering the OPA1 candidate region of 1Mb. 11 PAC clones constituting a minimal tiling path were selected for a sequence skimming of random subclones. Edited sequences were assembled into contigs and used for database searches and gene prediction analysis. Besides other EST and protein matches, we were able to identify a candidate gene most abundantly expressed in the retina. The open reading frame encoding a 960 amino acid polypeptide is split into 28 exons and spans more than 40 kb of genomic sequence. Upon sequence analysis in patients we identified mutations in 7 unrelated families with DOA from Germany, Britain and Cuba. The spectrum of mutations include deletions and insertions, a missense and nonsense alteration which segregate with the disease in the respective families. Since most mutations result in truncated polypeptides which probably represent null alleles we hypothesize that haploinsufficiency might be the predominant disease mechanism in DOA. Analysis of the N-terminal leader peptide of the deduced protein sequence suggests the OPA1 gene product is targeted to mitochondria and may exert its function in biogenesis and stabilization of mitochondrial membrane integrity. Thus, in similarity to Lebers optic atrophy, caused by mutations in the mitochondrial DNA, DOA might be due to an impairment of mitochondrial function caused by mutations in a nuclear gene.

Program Nr: 145 from the 2000 ASHG Annual Meeting

Homozygous null mutations of *ROR2* tyrosine kinase cause the autosomal recessive form of Robinow syndrome..

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Robinow syndrome (OMIM 268310) is a developmental disorder characterized by hypertelorism, short stature, mesomelic shortening of the forearms and forelegs and hypoplastic genitalia. The recessive form (RRS) has a high incidence of abnormalities of the vertebral column such as hemivertebrae and rib fusion. Some patients have cardiac malformations or facial clefting. Homozygosity mapping was used to localize the recessive RS gene in five consanguineous families. Further testing in the complete sample of 11 families revealed that all patients were homozygous for a set of 14 markers spanning 14 cM between markers D9S257 and D9S176 in 9q21.3-q2 (Zmax 11.25 at q = 0 for marker D9S1842). Haplotype sharing for seven markers was between three families from Turkey, enabled us to pinpoint the gene to a 4.9 cM interval. A partial YAC contig was then built for the RS critical region and used to map markers, ESTs and known genes .The ROR2 gene, encoding an orphan membrane-bound tyrosine kinase maps to this region (YACs 949E10 and 951D10) between markers D9S1781 and D9S197. Mutation analysis revealed several homozygous mutations in our cohort of RS patients. These mutations create premature stop codons and are predicted to result in non-functional proteins. Heterozygous (presumed gain of function) mutations in the ROR2 gene were also recently found to cause dominant brachydactyly type B (BDB). All reported BDB mutations are down stream of the RRS ones and do not involve the TK domain of the protein. We propose a model whereby complete loss of function of ROR2 causes Robinow syndrome, and gain or change of function causes BDB.

Program Nr: 146 from the 2000 ASHG Annual Meeting

Mutations of the homeobox gene *ALX4* in parietal foramina and cranium bifidum: a component of DEFECT 11 syndrome. *L.A. Mavrogiannis[1], A. Baxova[2], S. Kutilek[2], C.A. Kim[3], S.M. Sugayama[3], A. Salamanca[4], S.A. Wall[1], I. Antonopoulou[5], G.M. Morriss-Kay[5], A.O.M. Wilkie[1].* 1) Institute of Mol Medicine and Craniofacial Unit, Oxford Radcliffe Hospitals, Oxford, UK; 2) Dept of Paediatrics and Medical Genetics, Charles University, Prague, Czech Republic; 3) Genetica, Instituto da Crianca, Sao Paulo, Brazil; 4) Dept de Obstetricia y Ginecologia, Granada, Spain; 5) Dept of Human Anatomy and Genetics, University of Oxford, Oxford, UK.

Parietal foramina (PFM; OMIM 168500) are skull ossification defects occurring as isolated or syndromic malformations. Recently we identified mutations of *MSX2* (mapping to 5q34-q35) in non-syndromic PFM. However, PFM are also associated with multiple exostoses in the DEFECT 11 syndrome (OMIM 601224), caused by deletions of 11p11-p12 including the *EXT2* gene, indicating that a second PFM locus lies in this region. We generated a contig around *EXT2* and identified *ALX4*, the human orthologue of the mouse *aristaless*-related homeobox gene *Alx4*, 15 kb from the 3' end of *EXT2*. In mice, *Alx4*$^{-/-}$ mutations (including the *Strong's luxoid*, *lst*, allele) are associated with polydactyly and parietal bone defects, suggesting *ALX4* as a candidate gene for PFM. We characterized human *ALX4* which comprises 4 exons and extends over 45 kb, producing a 5-5.5 kb transcript that codes for a 411 aa protein; it shows 93.5% identity to murine Alx4. We report heterozygous mutations of *ALX4* in 17 affected members from four families segregating PFM, in which *MSX2* mutations had been excluded. These are two nonsense mutations (Q140X; Q246X) that truncate the DNA-binding paired-type homeodomain, and a R218Q substitution within the homeodomain, present in two unrelated families, including a 1 year old boy with a more severe cranium bifidum phenotype. The R218Q substitution is identical to the murine *lst* mutation, which was previously shown to abolish DNA binding. We conclude that haploinsufficiency of *ALX4* causes PFM, establishing DEFECT 11 as a true contiguous gene syndrome and highlighting a critical role for *ALX4* in skull ossification. The absence of limb defects in these patients points to relative differences in ALX4 dosage sensitivity between humans and mice during cranial and limb development.

Program Nr: 147 from the 2000 ASHG Annual Meeting

Etiology of autosomal recessive genetic disease in a child with noncarrier parents. *R.V. Lebo[1], L.R. Shapiro[2,3], E. Yosunkaya Fenerci[1], J.M. Hoover[3], J.L. Chuang[4], D.T. Chuang[4], D.F. Kronn[2,3].* 1) Center for Human Genetics, Boston University School of Medicine, Boston, MA; 2) Division of Medical Genetics, New York Medical College, Valhalla, NY; 3) Westchester Medical Center,Valhalla, NY; 4) Department of Biochemistry, Southwest Medical Center, University of Texas, Dallas, TX.

A child with maple syrup urine disease type 2 (MSUD2) was found to be homozygous for a 10 bp MSUD2 gene deletion on chromosome 1. Neither purported parent carries the gene deletion. Maternity was confirmed by analyses of 31 highly polymorphic loci: 15 unlinked autosomal loci and 16 syntenic chromosome 1 loci that span a genetic distance of 305 centimorgans including the MSUD2 gene. These results indicate the purported mother is the mother compared to a randomly selected woman with a likelihood exceeding 6,500,000,000 to 1. Then paternity was confirmed with a likelihood exceeding 2,900,000,000 to 1 based on results at the 15 autosomal loci not syntenic to the MSUD2 gene. Although the propositus is homozygous for maternally derived alleles at 15 of 16 loci syntenic to the MSUD2 gene, this child does not share paternally derived alleles at 7 of these syntenic loci. These data indicate that a de novo mutation prior to maternal meiosis I followed by nondisjunction in maternal meiosis II resulted in an oocyte with two copies of the de novo mutant allele. Fertilization by a sperm that did not carry a paternal chromosome 1 or subsequent mitotic loss of the paternal chromosome 1 resulted in the propositus inheriting two mutant MSUD2 alleles on two maternal number 1 chromosomes. The child also shares two different maternally derived chromosome 1 alleles at the D1S1598 syntenic locus as a result of two maternal meiotic recombinations on either side of this locus. The likelihood of finding a child with MSUD resulting from de novo mutation and uniparental disomy was calculated to be about 1 in 1,000,000,000 given a reported mutation frequency of 1.6 mutations/ diploid genome/generation, 200,000 genes in the diploid genome, 4 MSUD genes, and an estimated newborn UPD frequency for any single chromosome of 1 in 37,000.

Program Nr: 148 from the 2000 ASHG Annual Meeting

A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness, identifies the gene for Usher syndrome type 1C. *M. Bitner-Glindzicz[1,2], P. Rutland[1], K.J. Lindley[1,2], D. Blaydon[1], V.V. Smith[2], P.J. Milla[1,2], K. Hussain[1,2], J. Furth-Lavi[3], K.E. Cosgrove[4], R.M. Shepherd[4], P.D. Barnes[4], R.E. O'Brien[4], J. Sowden[1], M.J. Scanlan[5], S. Malcolm[1], M.J. Dunne[4], A. Aynsley-Green[1,2], B. Glaser[3].* 1) Institute of Child Health, London, WC1N 1EH,UK; 2) Great Ormond Street Hospital for Children NHS, Trust, London, WC1N 3JH, UK; 3) Hebrew University-Hadassah Medical School, Jerusalem, Israel; 4) Institute of Molecular Physiology and Dept. of Biomedical Science, Sheffield University, UK; 5) Ludwig Institute for Cancer research, New York Branch at Sloan Kettering Cancer Center, New York.

We have identified the gene for type 1C Usher syndrome by clinical and molecular analysis of two families with a contiguous gene deletion syndrome. Usher syndrome type 1 is a genetically heterogeneous disorder which comprises profound congenital sensorineural deafness with vestibular hypofunction and retinitis pigmentosa inherited in an autosomal recessive manner. It is an extremely handicapping disease which may be indistinguishable from non-syndromic deafness in the early stages. Clinical and molecular analysis of two consanguineous families in which affected children had a combination of hyperinsulinism, congenital deafness and enteropathy, revealed the presence of a recessive contiguous gene deletion on chromosome 11p15. FISH, STS mapping and candidate gene analysis enabled us to identify the gene responsible for type 1C Usher syndrome. This gene belongs to a class of genes not previously implicated in hearing loss. The work has important implications for the diagnosis and treatment of hyperinsulinism, enteropathies and also for the diagnosis of Usher syndrome and has shown that Usher type 1C is not a disorder confined to a single ethnic group as previously thought.

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Program Nr: 149 from the 2000 ASHG Annual Meeting

P63 mutations in the EEC, Hay-Wells, ADULT syndromes and in split hand/foot malformation reveals a genotype-phenotype correlation. *H. Van Bokhoven[1], J.A. McGrath[2], P. Duijf[1], J. Celli[1], B.C.J. Hamel[1], R. de Waal[1], A. Yang[3], F. McKeon[3], V. Doetsch[4], K. Vanmolkot[1], P. Propping[1], F. Gurrieri[1], G. Neri[1], M. Bamshad[5], H.G. Brunner[1]*. 1) Dept Human Genetics, 417, Univ Hosp Nijmegen, Nijmegen, Netherlands; 2) St. Johns Inst of Dermatology, St Thomas Hosp, London, UK; 3) Dept Cell Biology, Harvard Med School, Boston; 4) Dept Pharmaceutical Chemistry, UC San Francisco; 5) Univ Utah Health Sciences Center, Salt Lake City.

EEC syndrome is a developmental disorder characterized by Ectrodactyly, Ectodermal dysplasia, and facial Clefting. EEC syndrome has an autosomal dominant mode of inheritance and extreme clinical heterogeneity. We recently demonstrated that mutations in the p63 gene, a homolog of the archetypal tumor suppressor gene p53, cause EEC syndrome. An extended analysis revealed heterozygous p63 mutations in 26 of 30 unrelated EEC families, indicating that p63 is the major if not the only gene involved in EEC syndrome. Most of these mutations cause amino acid substitutions clustering in the DNA-binding domain. The constellation of the clinical signs in EEC syndrome shows some overlap with several distinct syndromes. Examples of such syndromes include Hay-Wells/AEC syndrome, Limb-Mammary Syndrome, ADULT syndrome, and Split Hand/Foot Malformation (SHFM). Mutation analysis of the p63 gene was performed to investigate the possibility of allelism of these EEC-related disorders. Causative p63 mutations were identified in AEC syndrome (8 mutations), ADULT syndrome (1 mutation), and SHFM (4 mutations). Except for one missense mutation in the DNA-binding domain in SHFM, the position and predicted effect of the mutations is clearly different from those in EEC syndrome. Among the newly identified mutations are missense, nonsense, and splice-site mutations. The functional consequences of the p63 mutations were investigated by RT-PCR, protein-modelling, transactivation assays and immunolocalization studies. These studies establish a phenotype-genotype correlation for p63 gene mutations. The fascinating new insights in p63 structure and function should pave the way for unravelling its role in the normal development of limb and ectodermal structures.

Program Nr: 150 from the 2000 ASHG Annual Meeting

Upregulation of *WNT-4* signaling causes dosage sensitive sex reversal in humans. *B.K. Jordan[1], M. Mohammed[2], S.T. Ching[1], X.-N. Chen[3,4], P. Dewing[1], N. Rao[2,3], B.R. Elejalde[5], E. Vilain[1,3]*. 1) Dept of Human Genetics; 2) Dept of Pathology and Laboratory Medicine; 3) Dept of Pediatrics, UCLA, Los Angeles, CA; 4) Cedars-Sinai Medical Center, Los Angeles, CA; 5) Medical Genetics Institute/Dept of Ob/Gyn, University of Wisconsin MCC, Milwaukee, WI.

Until recently, only transcription factors, including SRY, DAX-1, and SOX9, were known to play a role in human sex determination. Wnt-4, a member of the Wnt family of locally acting secreted growth factors, is the first signaling molecule shown to influence the sex determination cascade. In mice, a targeted deletion of *Wnt-4* causes the masculinization of XX pups. Therefore, the human homologue of *Wnt-4* is a strong candidate for sex reversal phenotypes in humans. The purpose of our investigations was to evaluate the potential role of *WNT-4* in human sex determination. Using cybercloning and 5' RACE PCR, we cloned and characterized *WNT-4*, the human homologue of murine *Wnt-4*. The WNT-4 protein shares more than 98% identity with its mouse homologue differing at only four C-terminal amino acids. We localized *WNT-4* by radiation hybrid mapping and Fluorescent *In Situ* Hybridization (FISH) to 1p35, a region previously shown to be involved, when duplicated, in XY sex reversal. We demostrated that an XY female with a duplication of 1p31-1p35 carries an additional copy of *WNT-4*, and we determined that *WNT-4* is overexpressed in the patient's fibroblasts. *In vitro* transfection assays demonstrated that *Wnt-4* upregulates *Dax-1* in a mouse Leydig tumor cell line (MLTC-1). Our results indicate that *WNT-4* is a novel gene resposible for XY sex reversal in humans. Control of *Dax-1* expression by *Wnt-4* supports our observation that overexpression of *WNT-4* results in XY sex reversal, a phenotype similar to that observed in patients with a duplication of DSS, a 160kb region containing *DAX-1*. These observations suggest that mammalian sex determination is sensitive to dosage at multiple steps in its pathway. Based on these data, we propose a new model for mammalian sex determination, consistent with all known defects in mouse and human sexual development, in which SRY inhibits DAX-1 via the WNT-4 signaling pathway.

Program Nr: 151 from the 2000 ASHG Annual Meeting

Mutations in the novel forkhead/winged-helix protein Scurfin cause neonatal diabetes, enteropathy, thrombocytopenia, and endocrinopathy syndrome, the human equivalent of the scurfy mouse. *R.S. Wildin[1], F. Ramsdell[2], J. Peake[3], F. Faravelli[4], J-L. Casanova[5], N. Buist[1], M. Brunkow[2]*. 1) Molec. & Med. Genetics, Oregon Health Sci Univ, Portland OR; 2) Celltech Chiroscience, Inc., Bothell WA; 3) Royal Brisbane Hospital, Herston Australia; 4) Laboratory of Human Genetics, Genova, Italy; 5) Hospital Necker-Enfants Malades, Paris France.

The syndrome of neonatal-onset **D**iabetes mellitus, **I**nfection, severe **E**nteropathy, **T**hrombocytopenia and anemia, **E**ndocrinopathy and eczema, and growth **R**etardation (**DIETER** Syndrome, a.k.a. OMIM 304930, 304790, 300063) is an X-linked recessive autoimmune disorder. Clinical variability is common, but it is usually fatal in infancy. Pathologic features include loss of pancreatic islets and erosion of the intestinal mucosa associated with focal inflammatory cell infiltration. Scurfy (*sf*) is an X-linked recessive neonatal lethal mouse with runting, infection, scaly skin, diarrhea and GI bleeding, anemia and thrombocytopenia, hypogonadism and cachexia. Leukocytosis and lymphadenopathy belie CD4+ CD8- T cells that are hyper-responsive to T cell receptor stimulation and secrete excessive cytokines. Human DIETER and mouse *sf* map to conserved regions. The mouse *sf* gene encodes scurfin, a novel protein with a forkhead/winged-helix domain (WH) that binds DNA. It is truncated upstream of the WH in *sf* mice. We hypothesized that DIETER is the human equivalent of *sf*, and sequenced the human ortholog in unrelated boys with DIETER phenotypes. Two had transitions at CpG dinucleotides that predict non-conservative substitutions in helix-3 (H3) and b-sheet-2 (S2) sub-domains, respectively, by comparison to the HNF3a and Genesis structures, implying a disruption of DNA binding. A third had a non-conservative substitution at an invariant amino acid C-terminal of Helix-2 (H2). A fourth harbored a short deletion/insertion mutation abolishing the termination codon. We conclude that DIETER is the human equivalent of mouse scurfy. These results have broad implications for the study of autoimmune disorders like type I diabetes and eczema, immune system modulation, transcription regulation in T cells, and protein:DNA interactions.

Program Nr: 152 from the 2000 ASHG Annual Meeting

EMSA studies define a GATA1/CP2 erythroid transcription complex in the uroporphyrinogen III synthase gene.
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Congenital erythropoietic porphyria (CEP), an autosomal recessive inborn error of heme biosynthesis, results from the markedly deficient activity of uroporphyrinogen III synthase (URO-synthase; EC 4.2.1.75). Phenotypic expression is highly variable from severe non-immune hydrops fetalis to mild cutaneous involvement in adults and the genotype can predict disease severity. Recently, we isolated and characterized the ~34 kb human URO-synthase genomic sequence and determined its organization and tissue-specific expression. The gene had two promoters that generated housekeeping and erythroid-specific transcripts with unique 5-untranslated sequences (exons 1 and 2A, respectively) followed by nine common coding exons (2B to 10). Northern blot, 5-RACE, and expression array analyses revealed that the housekeeping transcript was present in all tissues, while the erythroid transcript was detected only in erythropoietic tissues. The housekeeping promoter lacked TATA and SP1 sites, consistent with the observed low level expression in most cells, whereas the erythroid promoter contained GATA1 and NF-E2 sites for erythroid specificity. Sequence analysis of the erythroid-specific promoter in six CEP patients with a single undefined allele identified four novel mutations clustered in a 20 bp region: 1) a -70T to C transition in a putative GATA-1 consensus binding element, 2) a -76G to A transition, 3) a -86C to A transversion in three unrelated patients, and 4) a -90C to A transversion in a putative CP2 binding motif. Transfection of the -70C, -76A, -86A, or 90A luciferase promoter/reporter constructs into K562 erythroid cells resulted in 3 ± 1, 54 ± 3, 43 ± 6 and 8 ± 1% of normal construct activity, respectively. Electrophoretic mobility shift assays indicated that the -70C mutation altered GATA1 binding, and the -90C mutation altered CP2 binding. Thus, these four pathogenic erythroid promoter mutations impair erythroid-specific transcription, cause CEP, and define a novel functionally important GATA1/CP2 erythroid transcription complex for heme biosynthesis.

Program Nr: 153 from the 2000 ASHG Annual Meeting

EVALUATION OF PRENATAL DIAGNOSIS OF CONGENITAL ANOMALIES BY FETAL ULTRASONOGRAPHIC EXAMINATION IN EUROPE. *C. Stoll*[1], *M. Clementi*[2]. 1) Medical Genetics, HOPITAL DE HAUTEPIERRE, Strasbourg, France; 2) The EUROSCAN Working Group.

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Thanks to registries of congenital anomalies, a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of congenital anomalies by routine ultrasonographic examination of the fetus. All congenital malformations suspected prenatally and all congenital malformations, including chromosome anomalies, confirmed at birth were identified from the Congenital Malformation Registers, including 20 registers from the following European countries : Austria, Croatia, Denmark, France, Germany, Italy, Lithuania, Spain, Switzerland, The Netherlands, UK and Ukrainia. These registries are following the same methodology. The study was performed between July 1996 and December 1998, including 709,030 pregnancies. At delivery, 8126 babies/fetuses were diagnosed with abnormalities. The percentage of detection was variable for the diverse categories of congenital anomalies ; it was high for neural tube defects (NTD) 96.4% and 68.6 for spina bifida, but low for ventricular septal defect and for atrial septal defect (ASD), 6.7% and 7.9%, respectively. The detection rate was higher for multiply malformed children (for example four times higher for ASD). Detection rate varied between European countries according to the policies used : no routine scan, 1, 2 or 3 routine scans varying from 17.9% (no routine scan) to 55.6% (3 routine scans). The rate of pregnancy termination was high for central nervous system anomalies (54.5%) and chromosomal anomalies (53.1%) and low for renal anomalies (23.5%) and congenital heart defects (11.9%). Overall 25.0% of all pregnancies were terminated after prenatal detection of congenital anomalies. This study showed that many fetuses with major malformations can be identified prenatally in routine practice. Because policies, methods and techniques continually change, ongoing surveillance of prenatal diagnostic services is vital. »».

Program Nr: 154 from the 2000 ASHG Annual Meeting

Association between amniocentesis and congenital foot anomalies: report on the outcome of 4464 procedures performed between 11 and 19 weeks gestation. *G. Yoon[1], J. Chernos[1], B. Sibbald[3], R.B. Lowry[1,3], G. Connors[2], R. Simrose[2], F.P. Bernier[1]*. 1) Medical Genetics, University of Calgary, Canada; 2) Obstetrics and Gynecology, University of Calgary, Canada; 3) Alberta Congenital Anomalies Surveillance System (ACASS), Calgary, Canada.

Amniocentesis performed between 11+0 and 12+6 weeks of gestation is reported to be associated with an increased risk of club foot, with previously published frequencies as high as 1.3%. There is no reported data on the risk of foot anomalies when amniocentesis is performed between 13+0 and 14+6 weeks. In order to address this deficiency as well as confirm previous reports, a triple cohort retrospective study of the outcome of 4464 amniocenteses was carried out. The cohorts were defined according to gestational age at amniocentesis: 11+0 - 12+6 = early amniocentesis (EA), 13+0 - 14+6 = early mid-trimester amniocentesis (EMA) and 15+0 - 19+0 = mid-trimester amniocentesis (MA). Outcome measures were obtained from the Alberta Congenital Anomalies Surveillance System (ACASS) database by searching for children born with varus, valgus or other deformities of the feet as represented by ICD-9 codes 754.5,754.6 and 754.7. Foot anomaly rates were as follows: EA 11/962 (1.1%), EMA 11/2509 (0.4%) and MA 1/981 (0.1%). The differences between the EA and each of the EMA and MA cohorts were statistically significant; p=0.019 and p=0.003 respectively. There was no statistical difference between the EMA cohort and the MA cohort (p=0.11). The foot anomaly rate in the EMA group does appears to be intermediate, and the failure to detect a difference may be due to lack of power.

Conclusions: 1) Our foot anomaly rate of 1.1% for women who underwent EA is comparable to previously reported data, which validates the association as well as the methodology of this study. 2) The risk of foot anomaly is lower for EMA when compared to EA and appears to be similar to MA.

These results can be used to help couples make informed decisions regarding amniocentesis before 15 weeks gestation.

Program Nr: 155 from the 2000 ASHG Annual Meeting

First-trimester Down syndrome screening in twins. *F. Orlandi[1], D.A. Krantz[2], T.W. Hallahan[2], P.D. Buchanan[3], J.W. Larsen, Jr.[4].* 1) Centro Di Diagnosi Prenatale, Palermo, Italy; 2) NTD Laboratories, Huntington Station, NY; 3) GeneCare Medical Genetics Center, Chapel Hill, NC; 4) The George Washington University Medical Center, Washington, DC.

Recent studies suggest that first trimester Down syndrome (DS) screening using nuchal translucency (NT), free Beta hCG and PAPP-A is effective. In twin pregnancy, NT is measured on each twin separately and therefore separate risks can be provided. If biochemistry is added, a pseudo risk can be determined for each twin by adjusting the analyte values by the median level observed in twins. We studied 212 unaffected twin pregnancies (424 fetuses), between 10w4d and 13w6d GA, to determine if combining biochemistry with NT could improve the screening process in twins. Of the 424 fetuses, 21 (5%) were greater than the 95th %-tile for NT. The NT of at least 1 fetus was above the 95th %-tile in 17 of 212 pregnancies (8.0%). Of the 17 pregnancies, 13 pregnancies(6.1%) had one fetus and 4 pregnancies (1.9%) had both fetuses above the 95th %-tile. The median free Beta and PAPP-A levels were 1.96 and 1.64 MOM, respectively. The free Beta and PAPP-A SD (LOGe) was 0.4461 and 0.4400, respectively. Using a risk cut-off equivalent to a 35 year old, the observed pregnancy false positive rate was 13.2% using NT+age and 6.6% using NT+biochemistry+age. To determine if biochemistry improved screening performance we conducted a simulation trial using 10,000 sets of MOM values and a first trimester risk cut-off value of 1 in 250. For twin pregnancies discordant for Down syndrome, NT+Age resulted in a pregnancy false positive rate (FPR)of 10.2% and a detection rate (DR)of 80%. Adding in biochemistry resulted in a pregnancy FPR of 7.0% and an 80% DR. For twins concordant for DS, screening with NT+Age resulted in a FPR of 10.2% with a DR of 89%. Adding in biochemistry resulted in a pregnancy FPR of 7.0% with a DR of 94%. In first trimester DS screening of twins the addition of biochemistry to NT results in a reduction in the false positive rate at an equivalent or better detection rate thus indicating that the addition of biochemistry to NT will improve first-trimester DS screening performance of twin pregnancies.

Program Nr: 156 from the 2000 ASHG Annual Meeting

Cystic fibrosis prenatal screening program in a large HMO: Results from the first 12,000 screened women. *D.R. Witt, J. Phillipson.* Genetics Dept., Kaiser Permanente Medical Care Program Northern California, San Jose, CA.

After years of pilot studies, data analyses and controversy, cystic fibrosis (CF) population screening is becoming increasingly commonplace. This presentation describes the findings from a large CF prenatal screening program that has been implemented in the Kaiser Permanente Medical Care Program of No. CA. The report will discuss planning and logistical issues such as the efficient integration of CF testing with existing prenatal genetics screening for other diseases, staff training, development of effective pre-test educational materials and tracking/reporting systems for patients and specimens, and molecular laboratory requirements for high volume specimen throughput.

The Kaiser program has been in operation for approximately one year. It offers prenatal screening in a sequential format (female partner tested first; male partner tested only if woman identified as a carrier) to couples in which at least one partner is Caucasian. Acceptance of testing is high; approximately 12,000 women have been screened with an overall carrier identification rate of 1/28. Data will be presented on high-risk couples and prenatal diagnosis. Screening is done by a panel of 36 CFTR mutations and the poly T variant. Interesting results include the very high incidence of the R117H mutation and associated poly T variants, compound heterozygosity in asymptomatic women, counseling issues related to genotype-phenotype correlation, and comparison of molecular results in couples in whom both partners are Caucasian vs. "mixed" couples. Program costs and benefits will be reviewed. It is hoped that data from this program will assist other institutions and providers in the implementation of CF screening and guide decision-making for issues such as pre-test education, eligibility criteria for testing based on ethnicity, and mutation panel composition.

Program Nr: 157 from the 2000 ASHG Annual Meeting

Non-disclosing preimplantation genetic diagnosis for Huntington disease. *H.J. Stern, G.L. Harton, M.E. Sisson, S.L. Jones, L.A. Fallon, L.P. Thorsell, M.E. Getlinger, S.H. Black, J.D. Schulman.* Genetics & IVF Inst, Fairfax, VA.

Frequently, the diagnosis of Huntington disease (HD) is made in an individual when their children are already in reproductive age. At-risk individuals may elect to undergo presymptomatic testing, but must then face the significant emotional, medical, financial and social issues associated with both positive and negative test results. Others may not wish to pass on the HD gene mutation to their children but are not prepared to have presymptomatic testing and learn their genetic status. Many at-risk individuals would choose a method of genetic diagnosis that would assure them that children born would be unaffected by HD without revealing their genotype (non-disclosure). We report the implementation of a non-disclosing preimplantation genetic diagnosis (PGD) program which assists couples at high risk for offspring with HD to have healthy children without revealing the parent's genetic status. The process involves in vitro fertilization (IVF) with preimplantation embryo biopsy and molecular testing for HD if the parent carries a (CAG) expansion. Couples are informed that in order not to reveal any information from which they can infer their genetic status, they will not be informed of certain details of their IVF cycle. Patients are told only that HD-free embryos will be replaced in the uterus. Ten couples where one partner is at 50% risk for HD underwent 13 IVF and 2 frozen embryo transfer cycles as part of the non-disclosing HD PGD program at GIVF. In 8 of the 15 cycles, one partner was demonstrated to have a (CAG) expansion. In 11 cycles, embryos determined to be unaffected with HD were transferred, resulting in 5 clinical pregnancies, with 1 early loss, 2 ongoing pregnancies and delivery of a singleton and one set of twins. Both HD mutation carrier and non-carrier couples successfully achieved pregnancy. We believe non-disclosing PGD offers an important new reproductive alternative for at-risk individuals who desire to have healthy children, and that this approach should be reviewed, along with other relevant medical options, when counseling at-risk HD families.

Program Nr: 158 from the 2000 ASHG Annual Meeting

Recommendations for genetic counseling and screening of consanguineous couples and their offspring. *R.L. Bennett[1], A.G. Motulsky[1], A.H. Bittles[2], L. Hudgins[3], S. Uhrich[1], D. Lochner-Doyle[1], K. Silvey[4], C.R. Scott[1], E. Cheng[1], B. McGillivray[5], R. Steiner[4], D. Olson[1].* 1) Medical Genet.& Dept. OB/GYN, Univ. Washington, & Genet. Svcs. Section, WA State Dept. Health, Seattle, WA; 2) Centre for Human Genet., Edith Cowan Univ., Perth, Western Australia; 3) Div. Med. Genetics, Stanford Univ., Stanford, CA; 4) CDRC, Oregon Univ. & Oregon Health Sciences Univ., Eugene, Portland, OR; 5) BC Children's Hospital, Vancouver, BC.

There are few reports on how to advise and screen consanguineous couples, their pregnancies, and their offspring. A 1996 survey of U.S. medical geneticists and genetic counselors suggested that genetic screening practices and risk figures quoted for these couples and their offspring varied considerably (Genet. Med. 1999:1:286-292). A Consanguinity Working Group (CWG) consisting of experts in genetic counseling, medical genetics, biochemical genetics, public health genetics, genetic epidemiology, pediatrics, and perinatology met in Feb. 2000 to develop recommendations for genetic counseling and screening for consanguineous couples and their offspring, considering health services available in the U.S. and Canada. The goals were: 1. To provide reproductive options for family planning; 2. To improve pregnancy outcomes and identify prenatal screening options; 3. To reduce morbidity and mortality in the first years of life. Studies evaluating the risks for birth defects and mental retardation in this popualtion were assessed using MEDLINE and PubMed. Our recommendations were presented at regional meetings, and the CWG draft report is in the process of outside expert review for final adoption by the National Society of Genetic Counselors.

The CWG recommendations for genetic screening of consanguineous unions (related as 2nd cousins or closer) in a preconception, prenatal, and newborn setting are reviewed, as are specific genetic counseling issues (e.g., carrier testing in specific ethnic groups, stigma, cultural issues, family resources).

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Program Nr: 159 from the 2000 ASHG Annual Meeting

Routine prenatal testing for 22q11 deletion syndrome. *S. Manji, J.R. Roberson, A. Wiktor, S. Vats, P. Rush, S. Diment, D.L. Van Dyke.* Department of Medical Genetics, Henry Ford Hospital, Detroit, MI.

Congenital heart defects occur in about 1% of births, and 17-25% of cases of structural heart defects have a visible chromosome abnormality. In addition, microdeletion of band 22q11.2 is associated with a wide phenotypic spectrum including conotruncal heart defects, abnormal facies, thymic aplasia, cleft palate, hypocalcemia and learning problems. The incidence of 22q11 deletion syndrome is approximately 1 in 5-10,000 births, accounting for about 6% of all congenital heart defects, and making it one of the more common genetic conditions in the population. Fluorescent in situ hybridization (FISH) allows accurate detection of 22q11 deletion and can have significant implications for genetic counseling and intervention. We report on prenatal diagnosis by FISH of 22q11 deletion in fetuses identified on ultrasound with cardiac anomalies (excluding hypoplastic left heart and echogenic foci). Between January 1997 and May 2000 we identified 723 pregnancies in which congenital abnormalities were observed on ultrasound examination. Of these, 64 included cardiac abnormalities. Karyotype analysis on these 64 indicated that 18 (28%) had structural or numerical chromosomal anomalies, that could account for the cardiac defects. FISH testing for 22q11 deletion was performed on the remaining 46 amniotic fluid samples with normal karyotypes. Five of the 46 (10.9%) exhibited a 22q11 deletion. The cardiac abnormalities documented on the prenatal ultrasound included VSD, ASD, tetralogy of Fallot (TOF), AV canal defect and truncus arteriosus, which are all conotruncal heart defects associated with 22q11 deletion syndrome. Three of the 5 pregnancies were terminated, 1 infant was born prematurely at 24 weeks but did not survive and 1 full-term newborn underwent surgical correction of TOF. Four of the 5 fetuses were found to have de novo microdeletions and 1 was maternally inherited. Our experience suggests that routine FISH analysis for 22q11 deletion should be performed on all fetuses with cardiac defects and an otherwise normal G-banded karyotype. This allows families to make informed choices while providing clinicians accurate recurrence risk and outcome information.

Program Nr: 160 from the 2000 ASHG Annual Meeting

Fetal cell microchimerism in the thyroid. *B. Srivatsa[1], S. Srivatsa[2], K.L. Johnson[1], S.L. Lee[2], D.W. Bianchi[1]*. 1) Div of Genetics; 2) Div of Endocrinology, New England Medical Center, Boston, MA.

Background: It has been hypothesized that some autoimmune diseases in women may be "alloimmune" in nature, representing a chronic graft versus host response due to transplacentally acquired, immunologically active fetal cells. Thyroid diseases have a female predilection and postpartum exacerbation of autoimmune thyroiditis is common. The objective of this study was to determine the association between fetal cell microchimerism and thyroid disease in women.

Methods: Surgical specimens were obtained from 29 women who underwent thyroidectomy for various thyroid disorders (cases). The control group consisted of clinically and pathologically normal thyroids from 7 women who underwent autopsy for unrelated conditions. Medical records and pregnancy histories were reviewed. Archived paraffin embedded tissue blocks were cut into 5m sections. Fluorescence *in situ*hybridization (FISH) analysis was performed with probes specific for the X and Y chromosomes.

Results: The pathological diagnoses of the cases included: follicular adenoma (7), Hashimoto's thyroiditis (11), non-specific thyroiditis (7), carcinoma (papillary/undifferentiated) (7), and multinodular goiter (12). Some cases had multiple diagnoses. Twenty women had a history of a male pregnancy, 1 had an abortion and 8 had no history of a male pregnancy. Six of the control subjects had sons and 1 had an abortion. 16/29 (55%) cases and 0/7 (0%) controls had male cells in their thyroid sections. Male cells (range 1-165 per slide) were seen singly or in clusters in all diagnostic categories and were not limited to inflammatory thyroid diseases. In one goitrous subject with a son, the striking finding of fully differentiated male thyroid follicles closely attached to and indistinguishable from the rest of her thyroid was observed.

Conclusions: Microchimerism of presumed fetal origin can be demonstrated in surgically removed diseased thyroid specimens but not in normal thyroids at autopsy. Furthermore, fetal progenitor cells may be capable of differentiating into mature thyroid follicles in women.

Program Nr: 161 from the 2000 ASHG Annual Meeting

The presence of RhD gene sequences in RhD negative individuals. *J.M. DeMarchi, A. Merrill, H.D. Cook, B.A. Allitto.* DNA Diagnostic Laboratory, Genzyme Genetics, Framingham, MA.

The availability of molecular RhD analysis has clearly provided improved prenatal care for obstetric cases involving RhD incompatibility. The question remains, however, with what frequency can molecular analysis be an accurate predictor of prenatal risk? We isolated DNA from 746 specimens received in our laboratory for RhD analysis during 1998-1999 and examined sequences in the RhD gene on chromosome 1p34-1p36. Of the DNA samples tested, 257 originated from pregnant women who were serologically negative and sensitized to RhD antigen. We performed PCR amplification followed by separation of the products on agarose gels for molecular analysis. We amplified sequences in intron 4 and exon 10 of the RhD gene and co-amplified as controls, sequences shared by both the RhD and RhCcEe genes in exons 4, 5 and 7. Thirty of 257 DNA samples (11.7%) isolated from RhD negative women were positive for RhD gene sequences in exon 10. Of these, 7 were also positive for sequences in intron 4, 10 were weakly positive for sequences in intron 4, and the remaining 13 were negative. Twenty-nine of the 30 women had a singleton fetus that was positive for sequences in both RhD gene regions tested, and one had a fetus that was negative. At least one of the fetuses positive by molecular analysis was found to be RhD antigen-negative after delivery. When sorted by ethnicity, the RhD antigen-negative, gene sequence positive individuals were: 71% Black or African American, 14% Hispanic, 11% Caucasian, and 4% "other". Among the antigen-negative individuals whose molecular analysis was also negative, 56% were Caucasian, 25% Hispanic, 10% Black or African American, and 9% "other". These results show that for a substantial percentage of pregnant women who are sensitized to RhD antigen and whose fetuses are at risk for hemolytic disease, routine molecular analysis may not always provide an accurate assessment of fetal RhD status. The RhD negative status of the mothers is most likely due to mutations within the RhD gene, rather than absence of the gene as was originally considered, and the group most affected by this finding are individuals of African ancestry.

Program Nr: 162 from the 2000 ASHG Annual Meeting

Spontaneous abortions are reduced after Preimplantation Genetic Diagnosis of aneuploidy. *S. Munne, M. Sandalinas, S. Ernst, T. Escudero, S. Sadowy, J. Cohen.* Inst Reproductive Medicine/Sci, St Barnabas Medical Ctr, West Orange, NJ.

Selection of chromosomally abnormal embryos previously to embryo transfer in couples of advanced maternal age undergoing in vitro fertilization (IVF) has been demonstrated to reduce spontaneous abortions (Munne et al. 1999) and increase embryo implantation (Gianaroli et al. 1999). Those studies were retrospective and compared a population of patients undergoing preimplantation genetic diagnosis (PGD) after IVF with a control group receiving only IVF. Because many patients currently undergoing PGD had previous spontaneous abortions, a better way to assess the reduction in spontaneous after PGD is to compare the rate of miscarriage before and after PGD in the same group of patients. Patients undergoing IVF and PGD had one cell of each embryo biopsied, fixed, and analyzed by FISH with probes for chromosomes X, Y, 13, 16, 18, 21 and in a fraction of cases also with 15 and 22. The embryos classified as chromosomally normal for those chromosomes and with good embryo development and morphology were transferred on the same day of biopsy. The rate of spontaneous abortion on those patients that became pregnant was determine after at least 6 month of pregnancy and was compared with the rate of miscarriage in those same patients before PGD. Fifty-one patients undergoing PGD had 9 miscarriages out of 73 implanting embryos (12.3%) after PGD compared to 87 miscarriages out of 115 previous pregnancies (75.7%) before PGD ($p<0.001$). Of those patients 16 had three or more spontaneous abortions before PGD. After PGD these 16 patients had 20.8% spontaneous abortions (5/24 fetuses) compared to 89.2% (66/74 fetuses) before PGD ($p<0.001$). It is therefore concluded that PGD using probes for those chromosomes most involved in spontaneous abortions can significantly reduce embryo wastage in women of advanced maternal age and/or with recurrent miscarriages. Munne et al. (1999) Human Reprod 14 :2191-2199 Gianaroli et al. (1999) Fertil Steril 72:837-844.

Program Nr: 163 from the 2000 ASHG Annual Meeting

Preimplantation genetic diagnosis (PGD) for common aneuploidies: Reversing the relationship of PGD and IVF.

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Chromosome aberration is the most significant biological factor responsible for pregnancy failure and wastage. In this first randomized trial of its kind, we have demonstrated that selection of preimplantation embryos based on FISH analysis for seven chromosomes (13, 16, 18, 21, 22, X and Y) improved the background IVF pregnancy rate by eighty-four percent. Forty infertile patients undergoing IVF were randomized, with 21 assigned to the study group and 19 to the control group. All 8-cell embryos from the study group underwent assisted hatching followed by single blastomere biopsy. Eight-cell embryos from the control group underwent assisted hatching on day 3 without biopsy. All embryos underwent blastocyst transfer on day 5. Study and control groups were similar in age (35.1 vs 32.8 years); diagnosis; mean number of previous IVF attempts (1.1 vs 1.3); peak serum estradiol levels (2809 vs 2145 pg/mL); mean number of mature follicles (9.3 vs 7.7), implantation rates (i.e., number of gestations following embryo transfer) (25% vs 24%), and in proportion of patients experiencing spontaneous abortion. Mean number of preimplantation embryos undergoing FISH analysis was 6.8 per patient, with 80 percent euploid for the seven chromosomes analyzed. Eleven patients (52%) in the study group achieved a clinical pregnancy compared to six patients (31%) in the control group. Screening preimplantation embryos for common aneuploidies appeared to increase clinical pregnancy rates, when compared to background. The results of this study, still in progress, strongly suggest that all preimplantation embryos should undergo FISH analysis prior to transfer in order to enhance pregnancy outcome. These findings also point to an emerging shift in the relationship between IVF and preimplantation genetic diagnosis. FISH and other strategies for genome analysis will lead to a dependency of IVF on genetic analyses of preimplantation embryos rather than the reverse, which is the current paradigm.

Program Nr: 164 from the 2000 ASHG Annual Meeting

Highly-skewed X-inactivation patterns are associated with many patients presenting with idiopathic recurrent pregnancy loss. *L.M. Nelson[1,2], W. Branch[1], K. Ward[1,2]*. 1) Obstetrics and Gynecology, Univ of Utah Medical Center, Salt Lake City, UT; 2) Division of Perinatal Genetics,Univ of Utah Medical Center, Salt Lake City, UT.

Recurrent spontaneous abortion (RSAB) occurs in about 1-2% of women. Some causes, such as balanced translocations and structural defects, can be identified, but most RSAB etiologies remain unknown. Even without an identified reason for the RSAB, many women are treated with expensive and unproven treatments. Recently, several reports have suggested that a high degree of skewed X-inactivation may lead to an increased risk for RSAB. It has been hypothesized that this skewing may lead to an increase in the expression of X-linked recessive lethal genes. These studies suggest that about 15% of women with RSAB have a greater than 85% degree of X-inactivation skewing. We sought to investigate women with idiopathic RSAB to determine skewing in our patient population. We selected 57 women with RSAB who had a complete but negative RSAB workup including parental karyotypes. All women had at least 3 pregnancy losses. They were compared to 29 controls with a history of at least 2 live births. To determine the X-inactivation patterns in these women, we examined a highly-polymorphic CAG repeat in the human androgen receptor that is adjacent to Hpa II sites whose methylation predicts X-inactivation patterns. The repeat allows identification of both alleles about 90% of the time and the methylation will identify the X-inactivation pattern. The cases exhibited a high degree of skewed X-inactivation (29.8%) as compared to controls (3.4%) $p<0.0001$. These results suggest X-inactivation patterns are highly different in cases compared to controls. Our study showed almost a two-fold increase of patients with skewed X-inactivation compared to published results. This increase may be due to the highly-selected population we studied, which averaged one more loss than most studies. Our results agree that skewed X-inactivation plays an important role in RSAB. It appears to be the single largest cause of RSAB identified, and we suggest evaluation of idiopathic RSAB patients should include examination of X-inactivation patterns.

Program Nr: 165 from the 2000 ASHG Annual Meeting

X inactivation profile of Xp22 correlates with genomic content, banding pattern, and evolutionary history. *L. Carrel, K.C. Trevarthen, J.M. Dunn, H.F. Willard.* Dept Genetics, Case Western Reserve Univ, Cleveland, OH.

Previously we presented a first-generation X inactivation profile of the human X chromosome comprised of ~200 genes/ESTs assayed in a panel of somatic cell hybrids that retain human active (Xa) or inactive (Xi) Xs (Carrel, et al., PNAS 96:7364). These data demonstrated that genes on Xp were much more likely to escape inactivation than genes on Xq, presumably reflecting the autosomal evolutionary origin of the modern day Xp. To better understand both regional and chromosomal mechanisms controlling X-linked genes expression, we have focussed on Xp22, which appears to contain a large number of genes that escape inactivation. We have now expanded the dataset to include 28 additional genes in this region. Excluding the pseudoautosomal region, we have assayed a total of 67 genes/ETSs in Xp22. Of these, 52% escape inactivation. Overall, the propensity of genes in Xp22 to escape inactivation is 2.5 times higher than the X chromosome as a whole (based on analysis of 295 transcripts in total). Surprisingly, the distribution of genes within Xp22 is decidedly nonrandom. Most genes that localize to R bands (Xp22.1 and 22.31) are subject to inactivation (22 of 27 genes). In contrast, 24 of 27 genes that map to G bands Xp22.2 and p22.32 escape inactivation. Interestingly, 6 of 6 genes in the R band p22.33 escape inactivation; this may reflect its origin as part of the Xp/Yp pseudoautosomal region. These data suggest that the previously noted differences between Xp and Xq reflect a complex mosaic of genomic features, including gene-specific and regional elements, sequence composition and evolutionary history that together determine whether a gene will be expressed from the inactive X chromosome.

Program Nr: 166 from the 2000 ASHG Annual Meeting

XIST expression in human embryonal carcinoma lines: identification of novel sense and antisense transcripts.

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X-inactivation, the mammalian dosage compensation process, requires *XIST*, a functional RNA that is expressed exclusively from the inactive X. To study the initiation of X-inactivation in humans, we have analyzed *XIST* expression in embryonal carcinoma (EC) cell lines, which are the pluripotent stem cell population of germ cell tumors. N-Tera2-D1 (NT2) is derived from a testicular teratocarcinoma and expresses a low-level of *XIST* which can be seen by RNA-FISH as a pinpoint dot of expression in about 10% of cells, suggesting that the majority of cells have spontaneously differentiated. This expression pattern is reminiscent of undifferentiated mouse ES cells where *Xist* is expressed as a low-level, unstable transcript. However, unlike mouse, actinomycin experiments show that *XIST* in NT2 is relatively stable (t1/2 ~4 hours). RT-PCR at various sites across *XIST* show that transcription initiates midway through exon 1 and also extends downstream of *XIST* exon 8 in both NT2 and female somatic cells. By strand-specific RT-PCR, this low-level 3' transcript was found to be in the antisense orientation in NT2 but in the sense orientation in female somatic cells. An RNA antisense to *Xist*, *Tsix*, has been identified in mouse, and knockout studies indicate that it has a role in X-chromosome choice, possibly through *Xist* regulation. The mouse and human antisense transcripts are similar in that both initiate 3' of *XIST* and appear to be developmentally regulated with expression restricted to undifferentiated cell types. However, mouse *Tsix* expression is much more extensive, completely overlapping *Xist*, whereas in NT2 the antisense transcription only extends into the 3' end of *XIST*. The identification of antisense transcription associated with human *XIST* in early development suggests that it too may have a role in the early stages of X-inactivation. Further analysis of EC lines as well as other model systems will provide insight into its importance in the dosage compensation mechanism.

Program Nr: 167 from the 2000 ASHG Annual Meeting

Xp and Xq isochromosomes: mapping the functional centromere and testing Darlington's model of centromere misdivision. *H.F. Willard, A.W. Higgins, M.G. Schueler, K. Gustashaw.* Dept Genetics, Ctr Human Gen, Case Western Reserve Sch Med, Cleveland, OH.

To address the mechanisms of isochromosome formation and to localize the position of the functional centromere, we have determined the location of isochromosome breakpoints near the X chromosome centromere. To examine breakpoints in proximal Xp, we analyzed 20 cytogenetically monocentric i(Xq)s from Turner syndrome females by FISH, using a series of 26 probes derived from a large-insert clone contig from Xp11.21 to DXZ1. At least 10 different isochromosome breakpoints were documented. Only a single case appeared to break clearly inside the ~3 Mb DXZ1 array; 6 others broke within ~500 kb of DXZ1, within an extended region of diverged alpha satellite. We also examined breakpoints in proximal Xq in a series of 17 i(Xp)s generated in mouse/human somatic cell hybrids by the Pushmi-Pullyu counter-selection technique. These isochromosomes were analyzed by sequence-tagged site (STS) content using a large-insert clone contig from proximal Xq and by pulsed-field gel electrophoresis (PFGE). While many i(Xp)s contained multiple STSs from Xq and were thus structurally dicentric, 6 of the i(Xp)s lacked all Xq STSs, suggesting that they broke within or immediately adjacent to DXZ1. By PFGE analysis, 4 of the 6 showed clear rearrangements of DXZ1, indicating that the isochromosome formation event occurred within DXZ1. Restriction mapping was consistent with the centromere misdivision model proposed by Darlington in 1939 on the basis of his cytological observations in plants. Thus, while the majority of both i(Xq)s and i(Xp)s involve U-type sister chromatid exchanges occurring in proximal Xp and Xq, respectively, a subset of events involve centromere sequences directly. We further conclude from these studies that no sequences on Xp or Xq are uniquely required for proper segregation of the X chromosome, as the entirety of each chromosome arm is deleted in at least some isochromosomes. These deletion mapping data delimit the centromere locus on the X to DXZ1 and thus support the increasing evidence that alpha satellite sequences comprise the functional centromere on human chromosomes.

Program Nr: 168 from the 2000 ASHG Annual Meeting

Cytogenetic, molecular and sequence analyses of neocentromeric sequences from 9p: implications for activation of neocentromeres. *D.L. Satinover, S. Schwartz.* Dept of Genetics, Case Western Reserve Univ and Univ. Hosp, Cleveland, OH.

Although over 40 neocentromeres (neocens) have been reported in the literature, there has been little information has been generated on how these markers gain centromeric activity. To gain insight on how neocens become activated, our lab has focused on identifying the sequences involved in neocen activity. Previous studies in our lab have identified a common neocentromeric region (9p23) shared in two acentric chromosomes derived from 9p in unrelated individuals. Our current study was designed to develop a BAC contig across the neocentromeric region and to use immunofluorescence with centromeric proteins (CENPs C & E) in combination with BAC FISH to refine our neocentromeric regions. Our BAC contig has added an additional 29 STS and over 40 new BACs within this region of 9p23. Utilizing BACs within our contig, the neocentromeric region has been delineated to an ~350 kb region based on colocalization with the CENPs. To further refine our region, we have initiated chromatin immunoprecipitation (ChIP) experiments to identify neocen sequences associated with CENP-A. Preliminary data from these ChIP experiments reveal that the neocen sequence associated with CENP-A is no more than 260 kb. Analysis of these sequences reveals an AT content higher than in unique sequence DNA, equal to that of normal centromeric alpha satellite sequences. Within this region, we also have identified a higher than expected level of Long Terminal Repeat sequences (~20% LTRs). Our current studies of these two markers are significant in that they indicate that: (1) The neocentromere region can be sized cytogenetically to smaller than 350 kb and by ChIP analysis to no more than 260 kb; (2) A complete BAC contig of this region has been constructed and sequence from these BACs analyzed; and most importantly (3) We have shown that our neocen sequence associated with CENP-A has high levels of retrotransposable elements and an AT richness equal to that of alpha satellite. Our findings suggesting that generalized sequence composition and not primary sequence may be important in providing the necessary environment for neocen activation.

Program Nr: 169 from the 2000 ASHG Annual Meeting

Interaction Between a Human Homologue of the Silencing Regulator Sir2 and the Condensin Complex. *O.A. Cabello[1], K. Yokomori[3], B.R. Brinkley[1], J.W. Belmont[2]*. 1) Dept. Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX; 2) Dept. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) University of California, Irvine, CA.

Gene silencing is mediated by locus-specific or long-range condensation of chromatin, rendering it insensitive to the action of transcription factors. In S. cerevisiae, Sir2p is a determinant of life span and participates in the regulation of silencing at HM, telomere, and rDNA loci. At rDNA loci Sir2p is a component of the RENT complex that includes Net1 and Cdc14, but lacks any DNA binding component. It has been hypothesized that the anchoring element for RENT onto rDNA may possess catalytic activity and induce transcription repression by modulating the conformation of chromatin. Sequence comparisons of the core/catalytic domains of Sir2-related proteins indicate the existence of three Sir2-related gene subfamilies - HST1/Sir2, HST2, and HST3. We have identified a Sir2 homologue in mammalian cells, HSIR2L, that localizes to nucleoli and interacts with the condensin complex. The condensin complex is a heteropentamer required for high-order chromatin condensation at mitosis, but a role for condensin in transcription regulation has not been established. In this study, HeLa cells transfected with EGFP-HSIR2L were plated on glass coverslips, fixed and stained with antibodies specific to three condensin subunits. Deconvolution microscopy demonstrated that EGFP-HSIR2L co-localized with all three subunits at discrete foci concentrated in interphase nucleoli. In addition, one of the subunits, HCAP-H, co-localized with HSIR2L at additional scattered speckles throughout the nucleus. The nucleolar co-localization of HCAP-H and EGFP-HSIR2L was also observed in stable transfectants of EGFP-HSIR2L. The pattern of HCAP-H localization is identical to that seen in non-transfected cells. The specificity of interaction between HCAP-H and HSIR2L was established by coimmunoprecipitation. These results suggest that HSIR2L may be part of the RENT complex in human cells, and that the condensin complex may participate in transcriptional silencing by serving as an anchoring mechanism for RENT onto rDNA chromatin.

Program Nr: 170 from the 2000 ASHG Annual Meeting

The evolutionary origin of human subtelomeric homologies (...or where the ends begin). *C.M. Lese, A.H. Gross, J.A. Fantes, D.H. Ledbetter.* Dept Human Genetics, Univ Chicago, Chicago, IL.

Subtelomeric regions of human chromosomes, the 100-300 kb of DNA adjacent to the terminal TTAGGG repeat, are comprised of shared sequence homologies between subsets of chromosomes. Previous sequence analysis of subtelomeric regions demonstrated two subdomains, the *distal* subtelomeric sequence, characterized by short segments (<2 kb) of shared sequence homologies between many different chromosomes, and the *proximal* subtelomeric sequence, which contains longer segments (10-40 kb) of shared sequence homologies between fewer chromosomes. While developing a set of unique human telomere clones, we identified clones containing such shared homologies, characterized by the presence of hybridization signals on multiple telomeres by FISH. To examine the hypothesis that subtelomeric shared homologies result from ancestral genome duplications via unbalanced translocation events from the cognate telomere, we examined the evolutionary origin of 6 *proximal* subtelomeric clones that each showed hybridization to two human telomeres. ZOO-FISH analysis was carried out on metaphase spreads from chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), orangutan (Pongo paniscus) and macaque (Macaca fascicularis) using PAC63M14 (1p/8p), BAC131N4 (1p/8p), BAC136B17 (1p/4p), cos2003 (2q/8p), BAC113I2 (3q/5p), and BAC204M23 (4p/4q). For all clones, the origin of the duplications could be traced back to a single telomere, corresponding to one of the human orthologous sites. In Old World Monkeys, represented by macaque, all clones showed a single hybridization site, demonstrating that all duplications occurred < 23 million years ago, after the divergence of the Hominoids. Two clones showed a single signal only in macaque and two other clones showed a single signal in orangutan and macaque. Interestingly, the remaining two clones showed two signals in humans, but only one signal in chimpanzee and all other species tested; therefore, these duplications occurred since the divergence of humans and chimpanzees, < 3-5 million years ago. These results support the hypothesis that telomere rearrangements act as an evolutionary mechanism for gene duplication and creation of gene families.

Program Nr: 171 from the 2000 ASHG Annual Meeting

Frequent interchromosomal repeat array interactions between chromosomes 4 and 10: a model for subtelomeric plasticity. *P.G.M. van Overveld[1], R.J.F.L. Lemmers[1], G. Deidda[2], L. Sandkuyl[1], G.W. Padberg[3], R.R. Frants[1], S.M. van der Maarel[1]*. 1) Human and Clinical Genetics, LUMC, Leiden, The Netherlands; 2) National Research Council, Rome, Italy; 3) Department of Neurology, University of Nijmegen, Nijmegen, The Netherlands.

Chromosomal rearrangements occur more frequently in (sub)telomeric domains than in other regions of the genome, often resulting in disease. An intriguing example of disease-associated subtelomeric rearrangements is seen in facioscapulohumeral muscular dystrophy (FSHD), an autosomal dominant myopathy mapped to 4qter. FSHD is caused by partial deletion of a polymorphic array consisting of 3.3 kb-repeated elements. To further elucidate the behavior of this 3.3kb repeat array on chromosome 4 and its homologue on chromosome 10, we examined 208 healthy individuals. Although both repeat arrays have similar characteristics, they also display striking differences. For instance, the median repeat length of chromosome 4 is 16 kb larger than on chromosome 10, suggesting that chromosome-specific factors like telomere-length may be important for subtelomeric array sizes. The dynamic characteristics of the subtelomeric region are reflected in 24% of individuals carrying translocated repeat arrays of which 3% displaying somatic mosaicism for one of the alleles. Moreover, translocated repeat arrays on chromosome 4 tend to be more heterogeneous (i.e. composed of 4-type and 10-type units) than 4-type repeats on chromosome 10. This difference may be explained by the length difference in which larger 4-type alleles may allow double recombination more frequently. Furthermore, we identified as much as 3% of individuals carrying a FSHD-sized 4-type repeat. Finally, the repeat length distribution displays a periodicity of approximately 60 kb on both chromosomes which may reflect a higher order chromatin structure. This periodicity separates the repeat arrays on chromosome 4 in a normal and a premutation range. Overall, the observed dynamic characteristics of these homologous loci may serve as a model for subtelomeric plasticity.

Program Nr: 172 from the 2000 ASHG Annual Meeting

An AT-rich recombination hot-spot mediates the constitutional t(11;22) translocation. *B.E. Morrow[1], E. Spiteri[1], R.K. Pandita[1], S.R. Lalani[2], A. Shanske[3], R. Goldberg[1], L.G. Shaffer[2], L. Edelmann[1].* 1) Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY; 2) Dept Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 3) CTR Congenital Disorders, Montefiore Medical Center, Bronx, NY.

The constitutional t(11;22) translocation is the only known recurrent non-Robertsonian translocation in humans. Carriers of the constitutional t(11;22) translocation are at risk of having offspring with der(22) syndrome due to 3:1 meiotic nondisjunction events. We previously localized the recurrent t(11;22) translocation to a region on 22q11 within a low copy repeat, termed LCR22. LCR22s mediate rearrangements associated with congenital anomaly disorders on 22q11 such as velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS) and cat-eye syndrome. The t(11;22) breakpoint occurred in the same LCR22 as for the second most common distal deletion breakpoint in VCFS/DGS patients. FISH mapping studies showed that the t(11;22) translocation in unrelated carriers occurred within a similar region on 11q23, centromeric to the apolipoprotein gene cluster. Somatic hybrid mapping studies of two independent carriers allowed us to narrow the breakpoint on 11q23 to a 190 base pair AT-rich sequence motif. Novel junction fragments were identified in the derivative chromosomes by genomic Southern blot hybridization studies, using probes from 11q23, in a larger set of carriers and der(22) patients. To determine the molecular basis of the rearrangement, we cloned and sequenced the chromosome breakpoint junction in twelve unrelated individuals. The breakpoint occurred within complex AT-rich repeats on chromosomes 11 and 22. These data show that the AT-rich sequence motif serves as a recombination hot-spot. AT-rich repeats of varying sequence and length are present in the LCR22s associated with other 22q11 rearrangement disorders. We hypothesize that the AT-rich motif may be responsible for other rearrangements on 22q11 as well.

Program Nr: 173 from the 2000 ASHG Annual Meeting

Molecular characterisation of the constitutional t(11;22)(q23;q11) translocation and its affect on the expression of flanking genes. *A.S. Hill, N.J. Foot, T.L. Chaplin, B.D. Young.* Medical Oncology, I.C.R.F., London, UK.

The t(11;22)(q23;q11) is the most common recurrent non-Robertsonian constitutional translocation in humans and has been associated with a tenfold increase in the relative risk of breast cancer in balanced carriers. The 11q23 region also undergoes loss of heterozygosity in a variety of cancers such as breast, lung and ovarian. Using positional cloning we have mapped the t(11;22) breakpoints to specific Alu repeats on chromosomes 11 and 22 indicating that this event is due to an Alu-Alu recombination. In five balanced carriers the der(11) and der(22) breakpoints are all located within small regions of 32bp and 21bp respectively. EST sequences on chromosomes 11 and 22 were identified which located near or across the breakpoints. The inability to detect a transcript in a variety of tissues or isolate cDNA clones with the chromosome 11 ESTs indicates that they may not correspond to a real gene. The ESTs identified in chromosome 22 sequence appeared to correspond to 3 exons where the two 3 exons are located telomeric to the breakpoint. Northern analysis of various tissues and the t(11;22) cell lines (GM06229 and GM06275) detected transcripts ranging in size from 1.4kb to 9.0kb in testis RNA. However, attempts to clone the full length cDNA from a testis library were unsuccessful. 3 and 5 RACE using cDNA from GM06229 and GM06275 was used to look for chimeric transcripts but none were identified. If the chromosome 22 ESTs represent a real gene then it potentially has a large transcript specifically expressed in testis which may be alternately spliced. Established genes flanking the breakpoint on chromosome 11 include 3 apolipoprotein genes, APO AI, APO C3 and APO A4 (distal) and ZPR1 (proximal). ZPR1 is a zinc finger protein which binds to the tyrosine kinase domain of EGFR and is essential for cell viability. The possible deregulation of ZPR1 by the t(11;22) translocation and in cancer is being investigated.

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Olfactory receptor gene clusters mediate common chromosome rearrangements. *S. Giglio[1], N. Matsumoto[2], J. Fantes[2], V. Calvari[1], K.W. Broman[3], J.L. Weber[4], D.H. Ledbetter[2], O. Zuffardi[1]*. 1) Dept Med Genet, Univ Pavia, Italy; 2) Dept Human Genet, Univ Chicago; 3) Dept Biostat, J.Hopkins Univ; 4) Center Med Genet Marshfield Med Res Found.

The olfactory receptor (OR) gene superfamily is the largest in the mammalian genome. Human OR genes appear in clusters with 10 or more members located on almost all human chromosomes and with some chromosomes containing more than one cluster. We demonstrated that unequal crossovers between OR clusters are responsible for the formation of chromosome rearrangements. Several studies demonstrated that repeated sequences, located on the same chromosome at a distance of few megabases, predispose to homologous unequal recombination leading to chromosome microrearrangements. Deletions and reciprocal duplications can be mediated by duplicons having the same orientation whereas inversions seem to be due to unequal crossovers between duplicons with an inverted orientation.It seems likely that some macrorearrangements may also be mediated by the same mechanisms.Repeated sequences located on the same chromosome could be responsible for pericentric or paracentric inversions (according to their location on different chromosome arms or on the same arm) or for more complex rearrangements depending on the number of crossovers occurring between the two duplicons. Repeats located on different chromosomes could be responsible for translocations. Thus OR gene clusters might be the substrate for the formation of both intra- and interchromosomal rearrangements.We found that the common chromosome rearrangement inv dup(8p) (inverted duplication of 8p; estimated frequency 1:10-15000), associated with a severe malformation phenotype, is mediated by two closely spaced 8p-OR repeats. We also found that mothers of inv dup(8p) patients are all heterozygous for a benign inversion polymorphism involving the 8p-OR region.Thus this polymorphism seems to produce increased susceptibility to unequal recombination leading to the formation of a chromosome rearrangement.We also demonstrated that the deletion 8p22-p23.1, of whom several cases with similar cytogenetics breakpoints have been reported, is also mediated by the 8p-OR clusters.

Program Nr: 175 from the 2000 ASHG Annual Meeting

Delineation of Robertsonian translocations: Localization of satellite III sequences relative to breakpoints. *R. Bandyopadhyay[1], S.L. Page[3], S.A. Berend[1], C. Mcquillan[2], K.H.A. Choo[2], L.G. Shaffer[1]*. 1) Dept Molecular Human Genetics, Baylor Col Medicine, Houston, TX; 2) Murdoch Childrens Research Institute, Royal Children's Hospital, Melbourne, Australia; 3) Dept Molecular and Cell Biology, Univesity of California, Davis, CA.

Robertsonian translocations (Rob) are the most common rearrangements in humans, contributing significantly to genetic imbalance, fetal wastage, mental retardation and birth defects. Rob(14q21q) and rob (13q14q), which are formed predominantly during female meiosis, comprise the majority (85%) of all the Robs. Previous studies have shown that the breakpoints are consistently located within specific regions of the proximal short arms of chromosome 13, 14, and 21. The high prevalence of these translocations, the consistency in the location of the breakpoints and the fact that roughly 50% of cases occur de novo suggest that the sequences at or near the breakpoints confer susceptibility to chromosome rearrangement and the rearrangements occur through a specific mechanism. To determine the molecular basis of Rob formation, seven satellite III sequences from the short arms of the acrocentric chromosomes were identified, sequenced, and their chromosomal distributions determined. The physical order of the satellite III sequences that map to 14p was determined by fiber FISH analysis. Somatic cell hybrids were constructed using cell lines from four de novo rob(14q21q) carriers. For each de novo rob(14q21q), hybrids containing only the Rob and no other acrocentric chromosome, were identified and used in a PCR-based analysis to determine the presence or absence of the satellite III sequences on the translocations. Pulsed field gel electrophoresis (PFGE) demonstrated a patient-specific, novel junction fragment in the de novo rob(14q21q) somatic cell hybrids, which were not found in the parents. Southern analysis of this PFGE fragment has implicated two satellite III sequences in the formation of rob(14q21q). Overall, these studies are a significant step towards the cloning of the rob(14q21q) breakpoint and understanding the mechanism of formation.

Program Nr: 176 from the 2000 ASHG Annual Meeting

DNA replication pattern along a broad region that contains FRA7G, a common fragile site on human chromosome 7. *A. Hellman[1], S.W. Scherer[2], J. Skaug[2], D.I. Smith[3], B. Kerem[1].* 1) Dept Genetics, Hebrew Univ of Jerus, Jerusalem, Israel; 2) Dept Genetics, Hospital for Sick Children, Toronto, Ontario, Canada; 3) Dept Laboratory Medicine and Pathology, Mayo Foundation, Rochester, Minnesota.

Common fragile sites are chromosomal loci prone to breakage and rearrangement, considered to be part of the normal chromosome structure. The molecular basis for their fragility is not understood. Very late replication time has been observed for several common and rare fragile sites, hypothesized to lead to incomplete chromosome condensation during metaphase, resulting in fragility. Most (76/89) of the common fragile sites in the human genome, including the three cloned sites, FRA3B, FRA7H and FRA7G, map to early replicating R-bands. However, the sequences of the cloned sites revealed properties which resemble those of late replicating G-bands. Thus, fragile sites might be small G-bands regions embedded within large R-bands, transition regions between R/G bands, or regions with unique properties. To better understand the organization of replication domains along fragile, non-fragile and R/G transition regions, we analyzed the replication pattern along a ~1.5Mb contig that harbors the FRA7G and an R/G transition region (7q31.2/7q31.3). The replication analysis revealed that clones spanning the FRA7G region replicated relatively early in the S phase. However, high levels (30%-35%) of asynchronous replication were found, which are non allele-specific and can be formed in each cell division. This early and asynchronous pattern of the fragile region is in sharp contrast to the pattern of the adjacent early but synchrounsly replicating non-fragile region from 7q31.2, and the contiguous late and synchronously replicating region from 7q31.3. Importantly, the replication pattern of the fragile region resembles the replication pattern recently found along FRA7H (Hellman et al. 2000). Thus, the analysis of both FRA7H and FRA7G indicates that fragile regions might initiate their replication early in the S phase. However, due to intrinsic features which interfere in the replication fork movements some alleles might fail to complete the replication and condensation on time and thus, a fragile site will appear.

Program Nr: 177 from the 2000 ASHG Annual Meeting

Examination of epigenetic factors and gene-gene interactions influencing genetic susceptibility at chromosomes 7 and 15 for autistic disorder. *A. Ashley-Koch[1], M. Menold[1], K. Joyner[1], S. Mason[1], C. Poole[1], S. Donnelly[1], C. Wolpert[1], S. Ravan[2], R.K. Abramson[2], G.R. DeLong[1], M.L. Cuccaro[2], L. Von Wendt[3], C. McClain[4], H.H. Wright[2], J.M. Vance[1], J.G. Gilbert[1], M.A. Pericak-Vance[1]*. 1) Duke Univ Med Ctr, Durham, NC; 2) Univ of South Carolina, Columbia, SC; 3) Helsinki Univ Central Hosp, Helsinki, Finland; 4) Univ of New Mexico, NM.

Genomic screen and cytogenetic data have implicated 7q31-q32 and 15q11-q13 in genetic susceptibility for autistic disorder (AD). We previously showed suggestive evidence for paternal involvement in the chr 7 AD locus and maternal involvement in the chr 15 AD locus. In order to elucidate the epigenetic factors and gene-gene interactions influencing AD susceptibility at these loci, we examined 83 multiplex AD families. We observed excess paternal (p=0.008) but not maternal (p=0.37) IBD sharing at D7S640 and found suggestive evidence for increased maternal (p=0.06) but not paternal (p=0.34) IBD sharing at D15S217. Epigenetic effects at each locus were further examined by varying paternal or maternal heterozygote penetrances to maximize the heterogeneity lod score (hetlod) using GENEHUNTER-IMPRINTING. The peak 2pt hetlod assuming a traditional recessive model for chr 7 was 0.79 (a=0.15) at D7S495. Assuming 5% penetrance for paternal heterozygotes (P(++)=0;P(m+)=0.05;P(+m)=0;P(mm)=1) increased the hetlod to 1.12 (a=0.24)at D7S495. The peak 2pt hetlod assuming a recessive model for chr 15 was 0.67 (a=0.16) at D15S217. Incorporating 37.5% penetrance for maternal heterozygotes (P(++)=0;P(m+)=0;P(+m)=0.375;P(mm)=1) increased the peak hetlod to 1.10 (a=0.38) at D15S219. Next, we examined the effects of single versus two locus models. Single locus 2pt NPL analysis gave peak scores of 1.85 (p=0.03) at D7S640 and 2.05 (p=0.02) at D15S217. Single locus mpt analysis yielded peak scores of 2.47 (p=0.007) at D7S2527 and 1.74 (p=0.04) at D15S217. Analysis of the data under a two locus model via GENEHUNTER-TWOLOCUS gave peak 2pt and mpt NPL scores of 2.06 (p=0.02) and 1.98 (p=0.03), respectively. These data provide additional support for the involvement of chr 7 and chr 15 in AD susceptibility and suggest that these loci may be imprinted.

Program Nr: 178 from the 2000 ASHG Annual Meeting

Linkage Disequilibrium in Autism Families to Markers in the 15q11-q13 Autism Candidate Region. *J.S. Sutcliffe[1], and the Collaborative Linkage Study of Autism[1,2,3,4].* 1) Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN; 2) New England Medical Center, Boston, MA; 3) University of Iowa, Iowa City, IA; 4) University of North Carolina, Chappel Hill, NC.

Autism is a severe neurodevelopmental disorder characterized by repetitive or stereotyped behaviors and deficits in social interaction and language. Human chromosome 15q11-q13 has been implicated in autism susceptibility by observations of chromosomal duplications and linkage disequilibrium at candidate gene loci. In contrast to these findings, significant linkage of markers in the 15q11-q13 region in autism families has been detected by only one of five groups performing genome-wide linkage screens for autism loci (Bass et al. *Neurogenet*2: 219, 2000). The Collaborative Linkage Study of Autism detected multiple chromosome 15 abnormalities based on marker genotyping, but the most significant linkage in 15q was a maximum heterogeneity LOD of 0.51 at marker D15S975 in the initial study (CLSA. *Am J Med Genet* 88: 609, 1999). We have further analyzed this region using a test of linkage disequilibrium appropriate for sib-pair data sets (Martin et al. *Am J Hum Genet*61: 439, 1997), and found significant evidence for linkage disequilibrium at markers within and flanking a cluster of $GABA_A$ receptor subunit genes and the Angelman syndrome gene locus *UBE3A*; these markers include D15S122, D15S156 and a single nucleotide polymorphism at the *GABRB3*promoter (*P*values < 0.05). In all cases, statistically significant paternal-specific contribution to these scores was detected, possibly relating to genomic imprinting. No association was detected at other markers within the *GABRB3*locus, including 155CA-2 and marker GABRB3, at which other groups have detected linkage disequilibrium. Because multiple markers were analyzed, the scores were corrected for multiple tests. After correction, marker D15S122 remained statistically significant (*P*< 0.05). These data further support the genetic contribution of this region to autism susceptibility and suggest the involvement of genomic imprinting.

Program Nr: 179 from the 2000 ASHG Annual Meeting

Identification of a novel transcript in linkage disequilibrium with autism. *C.H. Wang[1,2,3], M.B. Miller[4], S. Ichikawa[2], R. Hillman[3], J.H. Miles[3]*. 1) Dept Neurology, Univ Missouri, Columbia, MO; 2) Dept Biochemistry, Univ Missouri, Columbia, MO; 3) Dept Child Health, Univ Missouri, Columbia, MO; 4) Dept Psychology, Univ Missouri, Columbia, MO.

Recent cytogenetic and molecular studies indicated that the chromosome 15q11-13 region is likely to contain an autism susceptibility gene. We have identified a meiotic recombination hotspot on chromosome 15q11-13 in children with autism. This recombination hotspot is located within a ~500 Kb interval between two closely spaced markers. Further physical mapping led to the isolation of a cosmid clone within this interval. Exon trapping from this cosmid yielded two authentic exons. These two exons are part of a novel 5.6 Kb transcript. In order to establish that this transcript is an autism susceptibility gene we performed single-stranded conformation polymorphism (SSCP) analysis to screen for disease-specific mutations in a large collection of autism samples. Primers were designed from the flanking intronic sequences to perform PCR for SSCP. One exon showed no sequence variation between 120 autism samples and 89 ethnically matched control samples. SSCP analysis of the second exon identified a 3-bp insertion/deletion polymorphism within the exon sequence. Fifty-nine of 120 (49%) autism samples and 19 of 89 control samples (21%) retained the homozygocity of the deleted isoform, a 2.3 times more prevalent in the autism samples. Fisher exact test showed the difference between the two groups was highly significant ($p<0.0001$). Further analysis using transmission disequilibrium test (TDT) showed the deleted isoform is in strong linkage disequilibrium with the autism locus ($c2 = 8.29$, $p = 0.016$). These results suggest that this novel transcript is a strong candidate for an autism susceptibility gene.

Program Nr: 180 from the 2000 ASHG Annual Meeting

Linkage disequilibrium studies of reading disability on 6p22. *D.E. Kaplan[1], T.W. Won[1], J. Ahn[1], D. Pauls[1], R. Olson[2], J. DeFries[2], F. Wood[3], G. Page[4], S.D. Smith[5], J.R. Gruen[1].* 1) Yale Univ Sch of Medicine, New Haven, CT; 2) Univ of Colorado, Boulder, CO; 3) Bowman Gray Sch of Medicine, Winston-Salem, NC; 4) Medical Univ of S Carolina, Charleston, SC; 5) Munroe Meyer Inst, Univ of Nebraska Med Center, Omaha, NE.

Several studies have consistently found a genetic locus for reading disability on 6p21.3-22. The reported locus sizes were 11cM spanning D6S422 (pter) through D6S291 (Fisher, AJHG, 64:146, 1999), 10cM spanning D6S109 through D6S306 (Grigorenko, AJHG, 60:27, 1997), and 5cM spanning D6S461 through D6S258 (Gayan, AJHG, 64:157, 1999). One additional study found evidence for allelic association with markers on 6p21 (AJHG, 65:A462, 1999). We cloned 10Mb of genomic DNA covering a large portion of the reported locus common to the three linkage studies with a sequence-ready BAC and PAC contig spanning markers pter-D6S422-D6S2241-cen, with no gaps. STR markers from the public domain were mapped and ordered by the clone contig. An additional 10 STR markers were developed from interspersed genomic sequence. From these markers a panel of 29 multi-allelic markers were chosen (average heterozygosity =0.73), that were evenly distributed through the 10Mb, and spaced apart about every 300kb. Using this marker panel we genotyped 420 subjects from 114 families where at least one offspring was referred for reading disability. Offspring (~150 sib-pairs for each phenotype) were phenotyped for discriminant word score, phonemic awareness, single word recognition, non-word reading, and orthographic coding. The data were analyzed with QTDT using orthogonal modeling as described by Abecasis (AJHG, 66:279, 2000), which accommodates quantitative traits, trios, single-parent families, and multiple offspring. We also performed linkage testing using the Haseman Elston algorithm with extreme phenotypes. Preliminary results show linkage disequilibrium (ChiSq=5.56, p=0.0183) in the presence of linkage (p=0.0026) with the same reading phenotype and the same marker. In addition, association was found with a different reading phenotype and another marker incorporating a maternal parent of origin effect (ChiSq=13.11, p=0.0003). (Support: NICHD-P01-HD21887).

Program Nr: 181 from the 2000 ASHG Annual Meeting

Search for schizophrenia genes in Finnish families reveal a locus on Chromosome 1q. *L. Peltonen[1,2], T. Paunio[1], J. Ekelund[1], T. Varilo[1], A. Parker[3], R. Martin[3], I. Hovatta[1], J.D. Terwilliger[4], J.S. Sinsheimer[2], J. Meyer[3], S. Maruti[3], J. Suvisaari[5], R. Arajärvi[5], T. Partonen[5], H. Juvonen[5], J. Lönnqvist[5].* 1) Department of Human Molecular Genetics, National Public Health Institute, Finland; 2) Department of Human Genetics, UCLA, Los Angeles, CA; 3) Millennium Pharmaceuticals Inc, Cambridge, MA; 4) Department of Psychiatry and Columbia Genome Center, Columbia University, New York, NY; 5) Department of Mental Health and Alcohol Research, National Public Health Institute, Finland.

We have earlier performed genome-wide scans for schizophrenia in Finnish families, identified from an internal isolate (Hovatta et al. 1999) as well as in affected sibpairs identified nationwide (Ekelund et al. 2000). Here, we analyzed 237 Finnish schizophrenia families with two or more affected individuals and (i) performed a genome-wide scan and (ii) carried out fine-mapping of the region of interest on chromosome 1q32. In statistical analyses of the combined study sample, two-point lod scores over 3 were obtained for loci on 1q and 5q. When the study samples from the internal isolate and from the whole Finland were analyzed separetely and homogeneity of the disorder was assumed, best lod scores in the internal isolate were obtained for markers locating on chromosomes 1q and 9q and in the families from the rest of Finland for those on chromosomes 1q and 5q. Further analyses of markers on chromosome 1 revealed that most of the evidence for linkage in the combined study sample was seen for markers around D1S3462 (Zmax=3.39), i.e. the same region identified in the previous study of sib-pairs from all over Finland. A novel gamete competition model also gave the strongest evidence for linkage for markers in this region. In the families from the internal isolate multipoint analysis using SimWalk2 showed evidence for linkage some 10-15 cM more centrally. Taken together our present and previous data, chromosome 1q contains a highly putative locus for a schizophrenia susceptibility gene but suggest genetic heterogeneity even in this population isolate.

Program Nr: 182 from the 2000 ASHG Annual Meeting

SNPs and bipolar disorder: discovery and genotyping. *P. Sklar[1,2], S. Bolk[2], V. Willour[5], P. Bennett[3], J.N. Hirschhorn[2], N. Burtt[2], G. Tsan[2], I. Jones[4], G. Kirov[4], M.J. Owen[4], N. Craddock[3], M. McInnis[5], D.R. DePaulo[5], E. Lander[2]*. 1) Psychiatry, Harvard Medical School, Boston, MA; 2) MIT/Whitehead Inst., MA; 3) University of Birmingham,UK; 4) University of Wales, UK; 5) Johns Hopkins Medical School, MD.

Family and twin studies have demonstrated a strong genetic basis for several bipolar disorder. Classical genetic linkage analyses have yet to conclusively define the relevant loci indicating that many genes with modest relative risk may underlie susceptibility to these disorders. We have identified coding region SNPs (cSNPs) in over 300 candidate genes for neuropsychiatric diseases from biochemical, pharmacological and historical candidates as well as candidate genes underlying areas of the genome implicated in genome scans. We have developed a method for genotyping hundreds of SNPs simultaneously using a fully generic tagged microarray. Using this method, SBE-TAGS, we genotyped over 100 SNPs (totalling over 5,000 genotypes) with 98-99% accuracy. SBE-TAGS has several attractive features: 30 or more genotyping reactions can be performed in a single microtiter well using unlabelled primers, highly multiplexed PCR reactions can be used as templates, the generic spotted tag array is easily generated, all reagents are commercially available, and determination of genotypes can be automated. Thus, SBE-TAGS brings the highly parallel capabilities of microarrays to bear on SNP genotyping in a way that is readily accessible to a wide range of research laboratories. Association analyses have been beset by several difficulties, ethnic stratification, population specific linkage disequilibrium between markers and causal variants, and inadequate statistical power to detect modest genetic effects. In order to address these problems we have preformed our association analyses in a large sample of bipolar parent-offspring trios using the transmission-disequilibrium test. Alleles showing nominal association in the initial sample were then tested for replication in an additional trio sample. The results of the first 50 SNPs studied will be presented.

Program Nr: 183 from the 2000 ASHG Annual Meeting

Further linkage evidence for a bipolar disorder susceptibility locus on 13q32. *C.Y. Liu[1,3], J.A. Badner[1], S.L. Christian[1], J.J. Guroff[2], S.D. Detera-Wadleigh[2], E.S. Gershon[1].* 1) Department of Psychiatry, University of Chicago, Chicago, IL, USA; 2) National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA; 3) National Laboratory of Medical Genetics, Hunan Medical University, Changsha, Hunan, P.R. China.

A region between D13S71 and D13S274 on 13q32 showed suggestive linkage to Bipolar disorder (BP) based on a genome scan using markers with an average spacing of ~6 cM and an average heterozygosity of ~60% (Detera-Wadleigh et al. 1999). In order to confirm this linkage and do fine mapping of the candidate gene, nine additional microsatellite markers allocated between D13S71 and D13S274 with average heterozygosity of ~86% were typed in the same sample collection. The strongest linkage evidence was detected by multipoint linkage analysis ASPEX around D13S779 - D13S225 with maximum LOD score of 3.7 under ASM II (P=0.000018). With the 9 new markers, the 95% confidence interval of the linkage region decreased to ~10 cM from ~14 cM. Association analysis with GASSOC detects potential linkage disequilibrium with several markers including D13S274 (ASM II, dominant, P=0.00075), D13S280 (ASM I, dominant, P=0.0041), D13S158 (ASM II, recessive, P=0.0050), and D13S793 (ASM II, recessive, P=0.0050). Most of these markers are clustered in a distal region of 13q32. Other evidence for a susceptibility gene for Schizophrenia (SZ) has been presented and replicated in the same region (Blouin et al. 1998; Brzustowicz et al. 1999). Our findings, in conjunction with prior studies, suggest that 13q32 may exert an important contribution to the genetic risk for BP and SZ.

Program Nr: 184 from the 2000 ASHG Annual Meeting

Fine-mapping of the Chromosome 12 Alzheimer Disease Locus using Family-Based Association Tests of Microsatellite Markers. *L. Bailey[1], W.K. Scott[2], J.M. Grubber[2], D. Hill[2], J.L. Hall[1], K. DeSombre[2], P.M. Conneally[3], G.W. Small[4], A. Roses[2], J.R. Gilbert[2], J.L. Haines[1], M.A. Pericak-Vance[2].* 1) Program in Human Genetics, Vanderbilt Univ, Nashville, TN; 2) Center for Human Genetics, Duke University Medical Center, Durham, NC; 3) Indiana University School of Medicine, Indianaplois, IN; 4) UCLA, Los Angeles, CA.

Several reports have established a wide interval (30 cM) on chromosome 12 as a likely location of a susceptibility locus for late onset familial AD. Initial follow-up in families with multiple members diagnosed with AD has extracted most of the potential linkage information in the region; thus the ability of linkage analysis to further refine the candidate interval is limited. Many candidate genes on chromosome 12 have been examined and none has been consistently associated with risk of AD. To narrow the region of interest, we have tested for association in a grid of 16 microsatellite markers, spaced on average 2 cM apart. These markers were genotyped in a set of 266 discordant sibships (DSPs; > 1 affected with late-onset (> 60) AD and > 1 unaffected) ascertained by Duke University Medical Center, UCLA, Indiana University, and the NIMH AD Genetics Initiative. Data were analyzed for linkage and association with AD using the Pedigree Disequilibrium Test (PDT), a test of linkage disequilibrium in general pedigrees. One marker, D12S1090 gave borderline evidence (p=0.06) for association. Based on this result, we tested for haplotype associations with AD in overlapping pairs of markers including D12S1090 and 2 proximal markers (D12S1090 - D12S1713 and D12S1713 - D12S2196) using TRANSMIT. The global chi-square test of association was significant for both D12S1090 - D12S1713 (p<001) and D12S1713 - D12S2196 (p<0.01). This data supports the localization of at least one AD susceptibility locus to chromosome 12 and suggests that examining haplotype transmission using microsatellite markers may prove to be a useful tool in helping to narrow candidate regions.

Program Nr: 185 from the 2000 ASHG Annual Meeting

Results and comparison of 2 genome-wide scans for age-related maculopathy (ARM). *M.B. Gorin[1,2], D.E. Weeks[2], Y.S. Conley[2,3], T.S. Mah[1], L.R Barnes[1], P.J. Rosenfeld[4], R.E. Ferrell[2].* 1) Dept. Ophthalmol., U. Pittsburgh SOM, Pgh, PA; 2) Dept. Human Genetics, U. Pittsburgh GSPH, Pgh, PA; 3) Dept. Health Promotion & Devel., U. Pittsburgh School of Nursing, Pgh, PA; 4) U. Miami SOM, Bascom Palmer Eye Inst., Miami, FL.

We have employed 2 genome-wide scans to identify susceptibility loci for ARM. The scans were done in collaboration with NHLBI Mammalian Genotyping Service (225 families) (1) and Center for Inherited Disease Research (CIDR) (195 families). Thirty individuals were included in both studies. This study addresses the differences in allele calls and the variability of results from successive genome-wide scans. The clinical methods have been described.(1,2) Each Center independently assessed error rates and we used our own error checking algorithms.(1) Linkage analysis for ARM susceptibility was performed with parametric and nonparametric models. Both Centers provided high quality data with small numbers of missing genotypes. We compared the alleles of the individuals who were genotyped twice. Absolute sizing of the alleles was problematic, requiring us to use center-specific allele frequencies for the linkage analyses. We previously reported linkage to chromosomes 9, 10, and 12 in our first genome-wide scan with further studies that supported the presence of linkage on chromosome 10.(1) Preliminary results from the CIDR scan indicate a different set of potential loci and limited support for linkages on chromosomes 10 and 12. There is a need for better calibration of genome-wide scans to achieve absolute sizes of allele calls, especially for association studies. The strategy of performing a single genome-wide scan and then focusing on a large number of positive and weakly positive, loci for additional genotyping can overlook potential loci that initially appear to be negative for linkage. Multiple genome-wide scans, though time-consuming and expensive, are essential for identification and confirmation of loci involved with complex and heterogeneous disorders. (1) H.M.G. '00, 9: 1329-49 (2) Ophthal. Genet. '98, 19:19-26. NIH EY09589, Res. to Prevent Blindness, Inc., NY, Eye & Ear Fndtn of Pgh (MBG) V Kann Rasmussen Fndtn (PJR).

Program Nr: 186 from the 2000 ASHG Annual Meeting

Evidence for linkage of psychotic bipolar disorder to 13q31 and 22q13. *J.B. Potash, V.L. Willour, T.H. Lan, J.R. DePaulo, M.G. McInnis.* Dept Psychiatry, Johns Hopkins Hosp, Baltimore, MD.

Objective:Recent linkage studies of schizophrenia and bipolar disorder have found overlapping evidence for potential susceptibility genes in four chromosomal regions10p12-13, 13q32, 18p11.2, and 22q11-13. We have previously exploited clinical features in an effort to reduce genetic heterogeneity in bipolar disorder families. Here we test the hypothesis that linkage evidence in these families for genes that increase susceptibility to both bipolar disorder and schizophrenia should be strongest in the families that are enriched for psychosis. Methods: Nonparametric linkage analysis using GENEHUNTER and ASPEX was performed on 65 bipolar disorder families containing 264 subjects with major affective disorder. Markers on chromosomes 10, 13, 18 , and 22 were analyzed in subsets of the pedigrees stratified by the number of affectively ill members with psychotic symptoms, defined as hallucinations and/or delusions. In 10 heavily psychotic families linkage analysis was extended to the whole genome. Results: A subset of 10 families which were the most heavily psychotic showed evidence for linkage to 13q31 with a NPL score of 3.33 (p=.0046) and evidence for linkage to 22q13 with a NPL score of 2.89 (p=.01). These were the two strongest signals in the genome for this set of 10 families. There was no evidence for linkage to 10p12-13 or 18p11.2. Conclusion: These data provide supportive evidence of psychosis genes on 13q31-32 and 22q13. Replication should be attempted in bipolar disorder and schizophrenia family samples enriched for psychotic bipolar disorder and schizoaffective disorder, as these phenotypes may be most closely associated with the putative susceptibility loci.

Program Nr: 187 from the 2000 ASHG Annual Meeting

A search for psoriasis genes in the Icelandic population. *A. Karason[1], A. Kong[1,3], M. Frigge[1], S. Snorradottir[1], J. Nahmias[1], E. Olafsdottir[1], H. Runarsdottir[1], A. Antonsdottir[1], K. Jonsdottir[1], V. Hauksson[1], R. Nicolae[1], J. Gudjonsson[2], S. Jonsdottir[1], J. Gulcher[1], H. Valdimarsson[2], K. Stefansson[1]*. 1) deCODE Genetics, Reykjavik, Iceland; 2) Department of Immunology, National University Hospital, 101 Reykjavik, Iceland; 3) Department of Human Genetics, University of Chicago.

Psoriasis is a chronic disease of the skin characterized by hyperkeratosis and inflammation. Psoriasis affects approximately 2% of western populations. The etiology is unknown, but it has a clear genetic component. It has a complex inheritance pattern that may reflect locus heterogeneity, epistasis (interacting loci), dynamic mutations, environmental factors, or a combination thereof. Here we report the results of a genome-wide linkage scan for psoriasis genes using 900 markers in 73 Icelandic families with 369 patients. We confirmed linkage to the major histocompatibility complex (MHC) with a non-parametric lod score of 11.0; no other locus of genome-wide significance was found. We genotyped our material with 35 markers in the MHC and observed an allelic association between psoriasis and several haplotypes within the MHC the strongest of which yielded a p-value of less than 10^{-9}. We also confirmed the association between the HLA-Cw6. We found about 67% of our population to carry Cw6 compared to a frequence in the general population of about 16%yielding a risk ratio of 10.4 for Cw6 carriers compared to non-carriers We then stratified our patients based on their Cw6 carrier status. Linkage analysis of the 2/3 of the patients carrying Cw6 resulted in suggestive linkages on several chromosomes and one linkage result on chromosome 19 which yielded a rise in LOD score from 1.59 to 4.33 . These data point to a significant epistatic interaction between a gene in the class I region of the MHC and a gene on chromosome 19. The penetrance of Cw6 carriers who do not also carry this extra at-risk allele is estimated to be 7.4% while the Cw6 carriers who also carry this at-risk allele have penetrance estimated to be 41%.

Program Nr: 188 from the 2000 ASHG Annual Meeting

Phenotypic characterization of the EH57.1 disease allele at psoriasis susceptibility locus PSORS1. *R.P. Nair[1], P. Stuart[1], T. Henseler[2], S. Jenisch[2], N.V.C. Chia[1], E. Westphal[2], N.J. Schork[3], J. Kim[4], H.W. Lim[4], E. Christophers[2], J.J. Voorhees[1], J.T. Elder[1]*. 1) Dept Dermatol, Univ Michigan, Ann Arbor, MI; 2) Depts Dermatol and Immunol, Univ Kiel, Germany; 3) Dept Biostat, Case Western Reserve Univ, Cleveland, OH; 4) Dept Dermatol, Henry Ford Hospital, Detroit, MI.

The psoriasis susceptibility locus PSORS1 has been mapped to the HLA region (6p21.3) and has been replicated in several data sets. We have fine mapped PSORS1 by means of association and haplotype analyses to a ~60 kb segment telomeric of HLA-C. Sixty-two physically mapped microsatellite markers covering the HLA region were genotyped in 339 parent-offspring trios in which the child had psoriasis. The transmission/disequilibrium test (TDT) showed significant linkage disequilibrium (LD) across the entire HLA region. Maximum LD was observed in an ~300 kb region extending from MICA to CDSN. A 1.2 Mb region of the central MHC containing this region was further analyzed in 478 trios. TDT analysis of individual markers and 2-5 marker haplotypes yielded a sharp peak of evidence for LD that spanned ~170 kb, centered at 100 kb telomeric of HLA-C. Recombinant ancestral haplotype analysis of the 170 kb region identified RH1, a 60 kb fragment of the ancestral EH57.1 haplotype. EH57.1 has been implicated previously in psoriasis. The RH1 allele is present in 61% of psoriatics in our sample. To further characterize the phenotype of RH1-associated psoriasis, we compared independent RH1+ve and RH1-ve psoriatics for their age at onset, severity of psoriasis, nail involvement, and arthritis. RH1+ve psoriatics (n=320) had a significantly lower mean age-at-onset distribution (19.7 vs. 23.9 years, P = .00125) than RH1-ve psoriatics (n = 226). No significant differences were seen for the other three variables. We conclude that heterogeneity exists in our sample; however, we cannot distinguish between allelic heterogeneity, locus heterogeneity, and the existence of nongenetic causes. RH1 does not contain any known genes, but contains several ESTs. The analysis of RH1 should greatly facilitate identification of the causative PSORS1 gene.

Program Nr: 189 from the 2000 ASHG Annual Meeting

First evidence of candidate loci for uric acid nephrolithiasis using an ideal genetic isolate. *N. Ombra[1], S. Casula[1], P. Forabosco[1], A. Angius[1], M.P. Pinna[1], E. Petretto[1], G. Colussi[2], E. Usai[3], R. Scarpa[3], P. Melis[1], M. Pirastu[1]*. 1) IGM-CNR, Alghero, Italy; 2) Ospedale Niguarda Milano, Italy; 3) Universita' di Cagliari, Italy.

In the last years isolated populations have been demonstrated to be an ideal tool for genetic mapping of complex diseases. We identified a small isolated village of Sardinia, founded by few individuals, with constant population growth, high endogamy and inbreeding, and where kidney stones (KS) has twice the prevalence than the general population. KS is a common multifactorial disease whose etiology is still unknown. Phenotype was characterized by: biochemically analyzed kidney stones; renal ultrasound scans; excretion parameters on 24 h urine collection; urinary pH (UpH), volume and sediment analysis. The majority of patients have a significant UpH reduction (UpH<5.5) as the only abnormal clinical parameter and uric acid as the principal component of kidney stones. We performed a multi-step genome-wide search in a deep-rooted pedigree connecting 48 affected relatives that we were able to reconstruct through archival records: in the initial stage 400 markers were typed in 14 closely-related affected subjects that we analyzed by parametric linkage and non-parametric IBD-sharing methods (using FASTLINK and SIMWALK2). Regions showing excess IBD-sharing ($p<0.05$) or LOD score >1 were further scrutinized in the whole pedigree. Allele frequencies were estimated from an independent population sample. We were able to identify new candidate regions on 10q and 20q. For the 10q region, a denser map of markers was also considered and significant linkage (peak LOD score=3.82) and excess IBD-sharing ($p<0.0001$) were obtained. Evidence of linkage disequilibrium ($p=0.004$) was also observed and haplotype reconstruction allowed us to identify a 1 Mb haplotype shared by 4 distantly-related affected subjects (peak NPL=7.6, $p=0.0002$) but not present in the general population. The remaining region on chromosome 20 (peak LOD score=1.4; peak NPL =2.1, $p=0.0322$) is currently being analyzed by typing additional markers to fine map the region of interest. These are the first candidate loci identified for KS of uric acid type.

Program Nr: 190 from the 2000 ASHG Annual Meeting

A new locus on chromosome 12p13.3 for pseudohypoaldosteronism type II, an autosomal dominant form of hypertension. *S. Disse-Nicodeme[1], J.M. Achard[2], I. Desitter[1], A.M. Houot[1], A. Fournier[2], P. Corvol[1,3], X. Jeunemaitre[1,3]*. 1) Unit 36, College de France, INSERM, Paris, France; 2) Service de Nephrologie, Hopital Amiens-Sud, Amiens, France; 3) Laboratoire de Genetique Moleculaire, Hopital Broussais, Paris, France.

Pseudohypoaldosteronism type II (PHA2) is a rare autosomal dominant form of volume-dependent low-renin hypertension characterized by hyperkalemia and hyperchloremic acidosis, but normal glomerular filtration rate. These features, together with the correction of blood pressure and metabolic abnormalities by small doses of thiazide diuretics, suggest a primary renal tubular defect. Two large loci have been previously identified on chromosomes 1q31-42 (PHA2A) and 17p11-q21 (PHA2B). We have now analysed a new large French pedigree, in which 12 affected members over 3 generations confirmed the autosomal dominant inheritance. Affected subjects had hypertension together with long-term hyperkalemia (range: 5.2-6.2 mmol/L), hyperchloremia (range: 100-109 mmol/L), normal plasma creatinine (range: 63-129 mmol/L) and low renin levels. Genetic linkage was excluded for both PHA2A and PHA2B loci (all lod scores Z<-3.2 at theta=0), as well as for the thiazide-sensitive sodium-chloride cotransporter gene. A genome-wide scan using 383 microsatellite markers showed a strong linkage with the chromosome 12p13 region (lod score Z=6.18, theta=0, at marker D12S99). Haplotype analysis using 10 additional polymorphic markers led to a minimum 13 cM interval flanked by D12S1652 and D12S336, thus defining a new PHA2C locus. Analysis of two obvious candidate genes (aENaC and Gnb3) located within the interval showed no deleterious mutation. In conclusion, we hereby demonstrate further genetic heterogeneity of this Mendelian form of hypertension and identify a new PHA2C locus, the most compelling and precise linkage interval described to date.

Program Nr: 191 from the 2000 ASHG Annual Meeting

Genomic Screen of 739 Sibpairs with Late Onset Alzheimer Diease. *M.A. Pericak-Vance[1], J. Grubber[1], L.R. Bailey[2], D. Hedges[1], S. West[1], B. Kemmerer[2], J.L. Hall[2], A.M. Saunders[1], A.D. Roses[1], G.W. Small[3], W.K. Scott[2], P.M. Conneally[4], J.M. Vance[1], J.L. Haines[2]*. 1) Duke University Medical Center, Durham, NC; 2) Vanderbilt University Medical Center, Nashville, TN; 3) UCLA Medical Center, Los Angeles, CA; 4) Indiana University Medical Center, Indianapolis, IN.

Two relatively small genomic screens [n=54, Pericak-Vance et al., JAMA 1997;278:1237-1241; n=230 Kehoe et al., Hum Mol Gen 1999; 8:237-245] have been published for late-onset AD. These have identified several potential regions for novel AD genes, including the as yet unidentified chromosome (chr) 12 gene. However, the modest sample sizes provided minimal power to detect effects that might arise in only a subset of families. We undertook the largest AD genomic screen to date using 466 families (from DUMC, VUMC, UCLA, IU and NIMH), a total of 739 affected sibpairs (ASPs) and an ~10 cM grid of microsatellite markers. There was some overlap in families with the previous screens but over 1/2 of these families have not been previously genotyped. We used 2-point and multipoint parametric lod score and non-parametric analyses. Six regions on six different chromosomes met our screening criteria of a lod score >1.00 in the overall dataset. Three regions confirmed previous results and three novel regions were identified. We also subdivided the dataset by autopsy status: Families with at least one autopsy confirmed case of AD (CONF, n=199) and families without any autopsy confirmation (UNK, n=267). Several new regions for AD came to light: in the CONF group on chromosomes 5, 10, and 18 (lods=2.23, 1.04 and 1.14, respectively), and in the UNK group on chrs 5 and 6 (lods=1.01 and1.31, respectively). We also examined these data using age of onset as the trait of interest and identified three additional novel regions. Despite being only 43% of the dataset, the CONF group gave much higher lod scores (8.98) than in the UNK group (3.03) for APOE. This suggests the possibility of etiological differences in these two groups. These results confirm that APOE has the largest genetic effect in AD and provides possible locations for other genes of lesser effect.

Program Nr: 192 from the 2000 ASHG Annual Meeting

ApoE and other loci affect age of Alzheimer's disease onset in families with PS2 mutation. *E.W. Daw[1], T.D. Bird[1,2], E.J. Nemens[2], D. Nochlin[1], G.D Schellenberg[1,2], E.M. Wijsman[1]*. 1) Univ Washington, Seattle, WA; 2) VA Medical Center, Seattle, WA.

Several kindreds of Volga German (VG) ancestry have a rare mutation in exon 5 of the PS2 gene on chromosome 1 which causes an autosomal dominant early-onset form of Alzheimers disease (AD). These families show a wide range in age-at-onset of AD (40-75 years with one inferred gene carrier who died unaffected at 89). Determining the cause of this range will further understanding of the biological mechanisms underlying AD. To examine whether the source of variation in onset is genetic, we performed a Monte Carlo Markov chain oligogenic segregation and linkage analysis on the 9 VG families (245 individuals) confirmed to have at least 1 affected PS2 carrier. This analysis estimated the effects of apoE and PS2 and the number and effects of unmapped loci affecting AD age-at-onset. In addition, a family-specific effect was estimated to account for some of the environmental variance. We estimate that the PS2 locus accounts for ~60% of the variance, apoE ~2%, and additional, unmapped, loci ~19%. Family-specific effects accounted for ~14%, and residual effects ~6%. The effects estimated for the apoE genotypes with e3 and e4 alleles were similar to estimates in our analysis of late-onset (LO) AD families (Daw et al. AJHG, 66:196). We estimate a 96% posterior probability of ³1 locus in addition to PS2 and apoE in the VG families (73% of ³2 loci). The largest two unmapped loci are estimated to contribute more to variation in AD onset (~13% and ~4%) than does apoE. Parameter estimates suggest these loci may be a subset of the loci estimated in our previous analysis of LO AD, with allele frequencies for the common allele of ~0.5 to ~0.8 and shifts of ~10 years in age-at-onset between the different genotypes. 11 markers around PS2 exclude linkage of these unmapped loci to this 60cM region. These results provide evidence that both apoE and other loci modify age-at-onset of AD caused by PS2 mutation, and that these other loci could also play a role in LO AD. The eventual mapping and cloning of these genes may be easier in the more genetically homogeneous VG families than in LO AD.

Program Nr: 193 from the 2000 ASHG Annual Meeting

Genome scan results of an independent set of Finnish affected sibling pairs with type 2 diabetes. *K. Silander[1], R. Watanabe[2], T. Valle[3], K. Mohlke[1], H. Stringham[2], K. Doheny[4], E. Pugh[4], R. Bergman[5], J. Tuomilehto[3], F. Collins[1], M. Boehnke[2], and The FUSION Study Group[1,2,3,5].* 1) NHGRI, NIH, Bethesda, MD; 2) Department of Biostatistics, University of Michigan, Ann Arbor, MI; 3) Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland; 4) Center for Inherited Disease Research, Johns Hopkins University School of Medicine, Baltimore, MD; 5) Keck School of Medicine, University of Southern California, Los Angeles, CA.

The Finland-U.S. Investigation of NIDDM Genetics (FUSION) study is an international collaboration that aims to map and positionally clone type 2 diabetes susceptibility genes. In an initial genome-wide scan we studied 719 affected sibling pairs from 478 Finnish families. This first set of families showed strongest evidence for linkage on chromosome 20, with weighted maximum lod scores (MLSs) of 1.99 at 17.5 cM, 2.04 at 56.5 cM, and 2.15 at 69.5 cM. An additional interesting locus was on chromosome 11 at 84 cM (MLS=1.75). We now have collected a second, independent set of 240 Finnish affected sib pair families (a total of 441 affected sib pairs). The two sets of patients were ascertained in a similar manner. However, the second set of patients has slightly shorter mean duration of disease (11.4 vs. 12.3 years) and lower mean fasting glucose values (9.3 vs. 10.4 mM). We have now completed genotyping of 382 markers for a genome scan on this second sample, in addition to typing 43 chromosome 20 markers, and have begun to analyze the resulting data. Our preliminary findings for the second sample provide additional evidence for a locus on 20p (MLS = 0.56 at 20.5 cM), but not for the other two chromosome 20 linkage peaks. When the two data sets are combined with additional affected siblings, removing the 10% of individuals with the self-reported earliest age of diagnosis increases the MLS to 3.58 at 17 cM. Statistical analysis of the remainder of the genome scan data is ongoing.

Program Nr: 194 from the 2000 ASHG Annual Meeting

Native American admixture and its relationship to diabetes in a Hispanic population of the Southwest. *C. Bonilla[1], E.J. Parra[1], C.L. Pfaff[1], K.G. Hiester[1], D.M. Sosnoski[1], S. Dios[1], F.O. Gulden[1], R.E. Ferrell[2], R.F. Hamman[3], M.D. Shriver[1]*. 1) Anthropology, Penn State University, State College, PA; 2) Dept. of Human Genetics, University of Pittsburgh, Pittsburgh, PA; 3) Dept. of Preventive Medicine and Biometrics, University of Colorado, Denver, CO.

Admixed populations can and should be used for gene mapping, since they exhibit higher levels of linkage disequilibrium (LD). Hispanics are heterogeneous admixed groups that originated when previously separated populations, namely Europeans, Africans and Native Americans, intermixed in the New World. We have studied the Hispanic population of the San Luis Valley (SLV) in Colorado, in terms of admixture and LD levels. Nine biallelic polymorphisms showing a frequency difference among parental populations greater than 0.3 were typed in 576 individuals of Hispanic origin. The Native American contribution, calculated by the weighted least squares method (Long, 1991), represents 31.6 ±1.9% of the SLV Hispanic gene pool. These results are in agreement with those obtained by Merriwether et al. (1997), who typed the same sample using six blood group antigens and five plasma protein markers and found an estimate of Native American admixture of 33.2 ± 2.4%. Like Merriwether and colleagues we found evidence of directional mating occurring in this population. Their analysis of mitochondrial DNA haplogroups showed an increased contribution of Native American females (85.1± 1.7%). We typed DYS199, a Y-chromosome marker, and observed predominantly European alleles. In addition, we tested the data for the existence of detectable LD between pairs of linked and unlinked markers. Since the samples were obtained as part of the SLV Diabetes Study, we compared diabetic cases and controls for significant differences in Native American ancestry. No association was found between diabetic condition and Native American admixture (cases: 28.5 ±1.5%, controls: 35.1±3.0%). This work provides a foundation for the use of Hispanic populations for admixture mapping of complex traits. This research is supported by grants from NIH/NIDDK (DK53958) and NIH/NHGRI (HG02154).

Program Nr: 195 from the 2000 ASHG Annual Meeting

Large-scale candidate gene association studies of type 2 diabetes. *J.N. Hirschhorn[1,2], D. Altshuler[1,3], C.M. Lindgren[4], M. Klannemark[4], M. Daly[1], M.-C. Vohl[5], J. Nemesh[1], C. Lane[1], S. Bolk[1], T.J. Hudson[1,5], L. Groop[4], E.S. Lander[1]*. 1) Whitehead Institute/MIT Center for Genome Research, Cambridge, MA; 2) Children's Hospital, Boston, MA; 3) Massachusetts General Hospital, Boston, MA; 4) University of Lund, Malmö, Sweden; 5) McGill University, Montreal, Canada.

Type 2 diabetes is a complex genetic disorder, with multiple genetic and environmental factors influencing disease risk. Association studies may provide a new, powerful approach to studying common, complex genetic disorders. We have therefore assembled the necessary tools to perform high throughput candidate-gene association studies of type 2 diabetes: polymorphisms in candidate genes, methods to type polymorphisms, and patient samples. As part of a concurrent project, polymorphism discovery is ongoing for over 300 candidate genes for diabetes. To utilize these single nucleotide polymorphisms (SNPs), we developed high throughput genotyping technologies based on single base extension (SBE). Finally, we assembled 333 parent-offspring trios with type 2 diabetes/abnormal glucose homeostasis, 1130 individuals from sibships discordant for diabetes, and 606 diabetic cases with matched controls.

To begin, we evaluated SNPs previously reported as significantly associated to type 2 diabetes or related sub-phenotypes. Our family-based design provides good power and is not susceptible to false-positive associations from ethnic admixture. Of 16 tested associations, we were only able to replicate one: PPARg Pro12Ala. This SNP showed transmission disequilibrium, with a 1.25-fold effect on diabetes risk that was further confirmed in independent samples. Critically, all published studies (including four “negative” studies) are consistent with our results (combined p value 0.00007). Our data thus support a modest but significant effect on diabetes risk for PPARg Pro12Ala. Our data also suggest the need for care to avoid false-positive and false-negative association studies. We are continuing to test novel SNPs for association to diabetes and other endocrine disorders: we thus far have preliminary evidence for association of a gene in the insulin signaling pathway with diabetes.

Program Nr: 196 from the 2000 ASHG Annual Meeting

A genome scan for circulating levels of the adipocyte-derived protein adiponectin reveals two quantitative trait loci with significant effects. *A.G. Comuzzie[1], T. Funahashi[2], G. Sonnenberg[3], L.J. Martin[1], A. Black[3], H. Jacob[3], J. Blangero[1], A. Kissebah[3].* 1) Southwest Fndn Biomed Res, San Antonio, TX; 2) Osaka Univ, Osaka, Japan; 3) Medical College of Wisconsin, Milwaukee, WI.

Here we present the first genetic analysis of adiponectin levels, a newly identified adipocyte-derived protein member of the collectin family of proteins. Recent work has suggested that adiponectin may play a role in mediating the effects of body weight as a risk factor for coronary artery disease [Circ. 100:2473, 1999]. For this analysis we assayed serum levels of adiponectin in 1100 adult individuals of predominantly northern European ancestry distributed across 170 families. All individuals were genotyped for 387 markers yielding an average map density of approximately 10cM. Robust linkage analysis was conducted using a variance component approach implemented in the program package SOLAR. Quantitative genetic analysis of adiponectin levels detected an additive genetic heritability of 46%. The maximum LOD score detected for adiponectin levels in a multipoint linkage scan was 4.06 ($p = 7.7 \times 10\text{-}6$) 35cM from pter on chromosome 5. The second largest LOD score (LOD = 3.2; $p = 6.2 \times 10\text{-}5$) was detected on chromosome 14 29cM from pter, and this signal persists (LOD = 2.1) in a subsequent oligogenic analysis conditional on the chromosome 5 QTL. The detection of a significant linkage with a QTL on chromosome 5 provides strong evidence for a replication of a previously reported QTL for obesity-related phenotypes detected in a genome scan in a French family study [Nat. Genet. 20:304,1998]. In addition, several secondary signals offer potential evidence of replications for previously reported obesity-related QTLs on chromosomes 2 (LOD = 2.7) [Nat. Genet. 15:273,1997; Nat. Genet. 20:304,1998], and 10 (LOD = 1.9) [Nat. Genet. 20:304,1998]. Not only do these results identify QTLs with significant effects on a newly described, and potentially very important, adipocyte derived protein, they also reveal the emergence of a consistent pattern of linkage results for obesity-related traits across a number of human populations.

Program Nr: 197 from the 2000 ASHG Annual Meeting

Genome scan for body mass index in the National Heart, Lung, and Blood Institute Family Heart Study. *M.F. Feitosa[1], I.B. Borecki[1], S.S. Rich[2], D.K. Arnett[3], P. Sholinsky[4], R.H. Myers[5], M.A. Province[1]*. 1) Div Biostatistics, Washington Univ Sch Medicine, St Louis, MO; 2) Wake Forest Univ, Winston-Salem, NC; 3) Univ Minnesota, Minneapolis, MN; 4) NHLBI, Bethesda, MD; 5) Boston Univ, MA.

Obesity is a risk factor for many chronic diseases including glucose intolerance, lipid disorders, hypertension and coronary heart disease. Even though body mass index [BMI = weight (kg) / height^2(m)] is a heterogeneous phenotype reflecting the amount of fat and lean mass, and body build and proportions, several studies have provided evidence for one or two major loci influencing this complex trait. Several adiposity phenotypes were assessed as part of the NHLBI-FHS including BMI, the waist-to-hip ratio, and a trunk to extremity skinfold thickness ratio. Here, we report the results of a genome scan in which linkage analysis was carried out using a variance components approach as implemented in SEGPATH (Province et al. 2000). Two complementary samples were studied: (a) 1,027 subjects distributed among 101 3-generational families with 404 markers typed by the Mammalian Genotyping Service (MGS), and (b) 1,328 subjects in 366 sibships with 243 markers typed by the Utah Molecular Genetics Lab. Analysis was carried out for each subsample, as well as for the combined sample in which the markers from each scan were placed on a common genetic map. There was evidence of linkage for BMI with chromosome 7 (137 cM) in each subsample: the lod scores were 2.1 (p=0.00073) and 3.2 (p=0.00007), for samples (a) and (b), respectively. The linkage is replicated by the consistent evidence from the mutually-exclusive subsets that were analyzed. Furthermore, evidence for linkage was enhanced in the combined sample with a lod score of 4.9 ($p < 10\text{-}5$). This signal is very near to the published location for the leptin gene (134.3 cM). Several other promising regions for BMI and other adiposity phenotypes with lod>2 also were identified. We will confirm these findings with the pending batch 2 MGS genotyped subjects, which will triple the sample size and approximately double the power. Completion of the genotyping will render the FHS a rich resource for mapping genes related to cardiovascular disease as well as adiposity.

Program Nr: 198 from the 2000 ASHG Annual Meeting

Fine mapping of the human familial combined hyperlipidemia locus on chromosome 1q21-q23 by utilizing the syntenic mouse locus on chromosome 3. *P. Pajukanta[1], J.S. Bodnar[2], R. Sallinen[3], L. Castellani[2], M. Wessman[3], A. Palotie[1], A.J. Lusis[2], L. Peltonen[1]*. 1) Human Genetics, UCLA, Los Angeles, CA; 2) Dept of Medicine, Dept. of Microbiology and Molecular Genetics and Molecular Biology Institute, UCLA; 3) Dept of Clinical Chemistry, Helsinki University Central Hospital, Finland.

Familial combined hyperlipidemia (FCHL) is one of the most common familial lipid disorders predisposing to premature coronary heart disease. Recently, we identified a novel locus for FCHL on human chromosome 1q21-q23 in Finnish FCHL families. Importantly, this human locus is syntenic to a mouse locus (Hyplip1) linked to combined hyperlipidemia on mouse chromosome 3 in the HcB-19/Dem mutant mouse. The interval containing the mouse gene Hyplip1 spanned originally about 3.5 cM. Here we restricted the critical interval of the Hyplip1 gene between markers D3Mit76 and D3Mit100, a distance of 1.5 cM. Further, we identified the borders of the syntenically conserved region between human and mouse by establishing a physical map of a bacterial artificial chromosome (BAC) contig across the Hyplip1 locus and by hybridizing a set of BACs to both human and mouse chromosomes by fluorescent in situ hybridization (FISH). In addition, BAC end sequencing from mouse BACs revealed several known genes that allowed the positioning of the human syntenic region to Hyplip1. In conclusion, we restricted the location of the mouse Hyplip1 gene to a region that exhibits synteny with human 1q21 and defined the synteny borders between human and mouse. Sequence analyses are now targeted to transcripts identified on this region to identify the mouse Hyplip1 gene and its human homologue.

Program Nr: 199 from the 2000 ASHG Annual Meeting

A genome-wide linkage study in autism. *J.D. Buxbaum, J.M. Silveman, C.J. Smith, M.A. Kilifarski, J.G. Reichert, B.A. Lawlor, M. Fizgerald, D.A. Greenberg, K.L. Davis.* Dept Psychiatry, Mount Sinai Sch Medicine, New York, NY.

Concordance rates for monozygotic and dizygotic co-twins of autistic probands indicate that there is a profound genetic component to autism and that inheritance is likely to be non-Mendelian. We have ascertained families with two or more individuals with autism or the related disorders of pervasive developmental disorder (PDD) and Aspergers syndrome. Blood samples were collected from affecteds, parents, and unaffected siblings and used for a genome-wide linkage study. A two-stage design was adopted in which genotyping is first carried out with markers at an average density of 10 cM in one set of families and markers demonstrating evidence for linkage passing threshold are then genotyped in an additional sample. In a sample of ca. 100 families, strong evidence for linkage was observed on chromosome 2q, with HLOD scores over 3 and approaching 4 in a subset of families with more severe illness.

Attempts to replicate other studies indicating linkage to chromosomes 7 or association with markers on chromosome 15 have also been carried out in the combined sample. Some evidence for linkage to 7q and association with markers on chromosome 15 were observed in the current study.

We are currently fine-mapping the region on chromosome 2 as a first step in identifying the gene in that region.

Program Nr: 200 from the 2000 ASHG Annual Meeting

Confirmatory evidence of linkage for autism to 7q based on combined analysis of three independent data sets.

S.L. Santangelo[1,2,3], C.L.S.A. Collaborative Linkage Study of Autism[2], J. Buxbaum[4], J. Silverman[4], M. Pericak-Vance[5], A. Ashley-Koch[5]. 1) Tufts School of Medicine, Boston, MA; 2) CLSA: Universities of Iowa, No. Carolina, and Vanderbilt; and Tufts University; 3) Harvard School of Public Health, Boston, MA; 4) Seaver Autism Research Center (SARC), Mt. Sinai School of Medicine, NY, NY; 5) Duke University, Durham, NC.

In order to follow up on an initial report of evidence of linkage for autism to 7q [MLS=2.5 @ 145 cM, IMGSAC, 1998], we jointly analyzed chromosome 7 data from our three independent ASP genome screens: CLSA (N=75), SARC (N=89), and Duke (N=83). The Duke sample gives a max multipoint MLS [Risch, 1990, with the possible triangle constraint of Holmans, 1993] of 2.5 @ 129 cM, while both the CLSA and SARC samples provide weaker evidence (1.4 @ 100 cM; 1.2 @ 155 cM respectively). When averaged across the studies, the MLSs are quite small across the entire region, which might suggest evidence against confirmation from the combined data. However, recent work [Vieland, Huang, & Wang, in press] has shown that when levels of heterogeneity vary across data sets, pooling data and averaging the scores without adequate allowance for inter-sample heterogeneity will tend to dramatically underestimate the true evidence for linkage when linkage exists. By contrast, in ASP data the sum across data sets of maximum MLSs is a robust approximation to a correct analysis allowing for inter-sample heterogeneity.

Summing the max MLSs from the three studies at 1cM intervals along a 68 cM stretch of 7q yields a total max summed MLS of 2.6 @ 130 cM with two additional peaks of 2.5 @ 100 cM and 2.4 @ 150 cM and with positive signal across the entire span. The p-value corresponding to a max MLS of 2.6 based on three data sets is approximately 0.004. Thus while the averaged MLSs did not appear to confirm linkage, basing our inference instead on the sum of max MLSs does appear to confirm a growing body of evidence for an autism gene in this region of 7q. However, precise localization of the gene within this 50cM region remains to be resolved.

Program Nr: 201 from the 2000 ASHG Annual Meeting

Correction of the dystrophic pathology in *mdx* mice by expression of the dystrophin isoform Dp260. *L.E. Warner, C. Dello Russo, R.W. Crawford, J.S. Chamberlain.* Dept. of Human Genetics, University of Michigan, Ann Arbor, MI.

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder caused by defects in the dystrophin gene. DMD is clinically characterized by severe, progressive muscle degeneration and weakness, which leads to wheelchair confinement and early death due to respiratory or cardiac failure. Dystrophin is part of a larger complex termed the dystrophin-associated protein (DAP) complex. The entire complex links the actin cytoskeleton to the extracellular matrix (ECM) and the loss of this complex results in contraction-induced injury and muscle degeneration in *mdx* mice and DMD patients. Dystrophin links to the ECM via a C-terminal connection to the DAP complex and binds the actin cytoskeleton via an N-terminal actin-binding domain. However, a second actin-binding domain has been identified *in vitro* in the central rod domain. Dp260, the retinal-specific dystrophin isoform, is deleted for the N-terminal actin-binding domain and the first 9 repeats of the rod domain, but retains the second actin-binding domain and the entire C-terminal region. We have constructed a murine Dp260 transgene to test its ability to reverse the dytrophic phenotype in transgenic *mdx* mice by establishing a mechanical link between dystrophin and the actin cytoskeleton. The level of transgene expression and extent of correction has been evaluated histologically, functionally, and by immunofluorescence. The initial lines studied showed a slightly mosaic pattern of expression; however, there appeared to be a correlation between expression of the transgene and protection of the fiber from damage as determined by lack of central nuclei. In addition, the Dp260 transgenic mice were protected from contraction-induced injury. Our results indicate that the Dp260 construct is able to at least partially prevent the development of dystrophic symptoms in transgenic *mdx* mice. Our ultimate goal is to identify the smallest dystrophin construct that has therapeutic potential since gene therapy for DMD will likely require the use of truncated dystrophin mini-genes to accommodate the limited cloning capacity of current viral vectors.

Program Nr: 202 from the 2000 ASHG Annual Meeting

Behavioral Characterization of Fxr2 mutant mice. *L.L. Kirkpatrick[1], K. McIlwain[1], C. Bontekoe[2], A. Peier[1], I. Nieuwenhuizen[2], N. Cheng[2], D.L. Nelson[1], B.A. Oostra[2], R. Paylor[1].* 1) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA; 2) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Fxr2 (Fragile X related -2) belongs to a small family of proteins that includes FMRP, the protein whose absence leads to fragile X syndrome. FXR1, the other member of this protein family, and FXR2 were initially identified in a yeast two hybrid screen for proteins that interact with FMRP. To assess the functional impact of Fxr2, Fxr2 was disrupted by homologous recombination in embryonic stem cells and homozygous knockout males, along with their wild-type male litter mates were evaluated using a test battery. The test battery included a basic neurological screen to assess simple motor/sensory function, open-field test for locomotor activity and anxiety-related responses, light-dark box for anxiety-related response, prepulse inhibition of the acoustic startle response to assess sensorimotor gating, acoustic startle habituation to assess sensorimotor adaptation, contextual and auditory conditioned freezing to measure conditioned fear, the hidden-platform test to study spatial learning, and the hotplate test for analgesia related responses. These studies are ongoing but some preliminary findings suggest that the Fxr2 knockout mutants may have different anxiety-related responses. The findings from this project will provide important insight into the possible role of Fxr2 in central nervous system function. Double knockouts of Fmr1 and Fxr2 should provide data regarding the likelihood that Fxr2 compensates for loss of FMRP in humans with fragile X syndrome.

Program Nr: 203 from the 2000 ASHG Annual Meeting

Differential reactivation of the FMR1 gene in fragile X patients cell lines. *G. Neri[1], M.G. Pomponi[1], R. Pietrobono[1], P. Chiurazzi[2], B.A. Oostra[3].* 1) Istituto di Genetica Medica, Universita' Cattolica, Rome, Italy; 2) Istituto di Pediatria Medica,Preventiva e Sociale, Universita' di Messina, Messina, Italy; 3) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands.

Fragile X syndrome is the leading cause of heritable mental retardation, with a prevalence in the general population of 1:4000 males. Most fragile X patients have an amplified CGG repeat in the promoter of the FMR1 gene, which becomes abnormally methylated and inactive. We demonstrated that reactivation of the fully mutated gene can be achieved by treating patients' lymphoblasts with the demethylating drug 5-azadeoxycytidine (Hum Mol Genet 7:109-113, 1998) and, although at very low levels, also with 4-phenylbutyrate or butyrate (histone hyperacetylating drugs). However, when 4-phenylbutyrate or butyrate were used with 5-azadeoxycytidine, a marked synergistic effect was observed (Hum Mol Genet 8:2317-2323, 1999) thus confirming that DNA hypermethylation and histone deacetylation are sequential steps leading to the transcriptional silencing of the fully mutated FMR1 gene. We are now determining how the different FMR1 reactivation levels obtained in several cell lines correlate with the size of the CGG expansion and the extent of the promoter hypermethylation. In fact we noted that cells harbouring a longer CGG expansion seem to be reactivated less efficiently. Therefore we have optimized a bisulphite-PCR protocol to assess the methylation of individual CpG sites in the FMR1 promoter before and after various drug treatments. Preliminary data indicate that transcription is restored after demthylation of few specific CpG sites. We also intend to verify whether treatments with histone hyperacetylating drugs alone determine any demethylation of the FMR1 promoter. These studies will assist in the optimization of potential pharmacological strategies for in vivo reactivation of the FMR1 gene. Supported in part by a grant of the FRAXA Foundation and by Telethon grant E-243.

Program Nr: 204 from the 2000 ASHG Annual Meeting

Fragile X premutation alleles are associated with reduced FMRP levels due to a reduction of message translatability. *A. Kenneson, F. Zhang, S.T. Warren.* Departments of Biochemistry, Genetics, and Pediatrics, Emory University, Atlanta, GA, and Howard Hughes Medical Institute, Atlanta, GA.

Fragile X syndrome, characterized by mental retardation and macroorchidism, is typically the result of a large (>200 repeats) in the trinucleotide CGG tract in the 5' UTR of the FMR1 gene, which reults in the absence of the FMR1 gene product, FMRP. Premutation alleles have between 50 and 200 repeats, are meiotically unstable, and generally do not have an impact on phenotype. We have developed a sensitive and quantitative assay for the accurate measurement of FMRP levels in EBV-transformed human B lymphoblastoid cell lines, and examined FMRP levels in cells from three groups: normal males with repeats in the normal range, normal males with premutation alleles (105 to 130 repeats), and mildly-affected males with premutation alleles (140 to 190 repeats). We detected a 20% decrease in FMRP levels in cells from unaffected premutation carriers(mean=0.171 normalized to control protein eIF4e, se=0.004, N=4) compared to cells with repeats in the normal range (mean=0.218, se=0.008, N=7). Cells from affected individuals with the larger premutations had a 40% reduction in FMRP (mean=0.128, se=0.003, N=2) compared to cells with normal repeat numbers. This is the first report of reduced FMRP levels associated with premutations in unaffected individuals. In addition, the difference in FMRP levels between the affected and unaffected premutation carriers suggests that there is a critical level of FMRP required for normal cognitive function, with smaller premutations being associated with an apparently sub-clinical reduction of FMRP. The reduced FMRP level of premutation alleles is due to a reduction in translatability of FMR1 messages as mRNA levels are increased compared to that of normal alleles. These data suggest that a feedback mechanism may exist which compensates for the reduction in translatability through the larger CGG tracts by increasing message level. Moreover, the observation of a significant reducation of FMRP in cells of apparently normal premutation carriers re-opens examination of anecdotal reports of penetrance in carriers, perhaps in conjunction with modifying genes.

Program Nr: 205 from the 2000 ASHG Annual Meeting

Nonstop decay: A novel mRNA decay pathway. *P.A. Frischmeyer, K. O'Donnell, H.C. Dietz.* HHMI and Inst of Genet Med, Johns Hopkins Univ SOM, Baltimore, MD.

Modulation of mRNA stability represents a powerful mechanism to regulate the level and fidelity of gene expression. Transcripts containing premature translation termination codons (PTCs) are recognized and degraded by the nonsense mediated mRNA decay (NMD) pathway. Although NMD is ubiquitous among eukaryotes, little is known about its basic mechanism. One question that remains is whether NMD results from translation termination in an improper context or a failure to terminate in the proper context. Both wild type (WT-gus) and nonsense-containing (PTC-gus) mini-gene constructs were generated. Transcripts derived from the nonsense form of the mini-gene were 5-fold less stable than those derived from the WT-gus construct. To investigate the importance of proper translational termination in NMD and normal RNA stability, the bona fide termination codon and all in-frame termination codons in the 3UTR of PTC-gus and WT-gus were removed to create constructs termed PTC-NoTer-gus and Nonstop-gus, respectively. PTC-NoTer-gus transcripts were degraded as efficiently as the parent nonsense form from which it was derived, suggesting that NMD does not require recognition of the bona fide termination codon in its proper context. Nonstop-gus transcripts were as labile as their nonsense counterparts, suggesting a critical role for translational termination in normal mRNA stability. In transcripts that lack all termination codons, the ribosome may translate through the 3' UTR and poly(A) tail, disrupting PABP binding and hence the closed loop conformation and stability of the mRNA. Indeed, addition of a stop two codons away from the site of poly(A)tail addition was sufficient to restore the stability of Nonstop-gus transcripts to near-wild type levels. We show that nonstop decay is a novel mRNA decay pathway that is dependent on translation but independent of trans-effectors essential for NMD. It is interesting to speculate about potential endogenous origins of nonstop transcripts. A search of the GenBank database reveals that many human cDNAs contain consensus polyadenylation signals in their coding region. We propose that nonstop decay may clear the truncated transcripts that result when premature polyadenylation signals are used.

Program Nr: 206 from the 2000 ASHG Annual Meeting

Unusual X-Linked SCID Phenotype due to Mutation of the Poly-A Addition Signal of IL2RG. *A.P. Hsu[1], E.J. Tsai[1,2], S.M. Anderson[1], R.E. Fischer[1], H. Malech[3], R.H. Buckley[4], J.M. Puck[1]*. 1) Genetics & Mol Biol Branch, NHGRI, NIH, Bethesda, MD; 2) HHMI-NIH Research Scholars Program; 3) Lab Host Defenses, NIAID, NIH, Bethesda, MD; 4) Dept of Pediatr, Duke University Sch of Med, Durham, NC.

Severe combined immunodeficiency (SCID) is a syndrome of profound cellular and humoral immune defects. The most common, X-linked form (XSCID) has no T cells and nonfunctional B cells, resulting from defects in the interleukin-2, -4, -7, -9 and -15 signaling through the common gamma chain (gc) cytokine receptor encoded by IL2RG. Patients with XSCID present in their first year with failure to thrive and infections that fail to resolve. Currently HLA-matched or haploidentical T-cell-depleted bone marrow transplantation (BMT) rescues up to 90% of promptly diagnosed patients. A male with sporadic SCID unexpectedly failed to engraft after 4 haploidentical BMTs, 2 from each parent. Despite poor growth and chronic infected skin rashes, he has survived to age 8 with immunoglobulin replacement and agressive management of infections. He has had 200-500 T cells/ul with decreased mitogen proliferation and no specific antigen responses; these cells may have been responsible for rejecting BMTs. XSCID was suspected based on maternal skewed T cell X inactivation. A B-cell line from the patient lacked gc cell surface expression; Northern blot revealed a low amount of a 4 kb mRNA (nl IL2RG mRNA 1.8 kb). No DNA mutations were found within exons, flanking splice sites, or PCR products spanning the entire 4 kb gene. A cDNA library from the patient's B-cell line yielded >95% IL2RG clones with a cDNA1451 A-G transversion changing the final A of the AAUAAA polyadenylation signal to AAUAAG. This caused transcription to continue through the poly-A site, accounting for observed large mRNA size. Both the B-cell line and isolated CD34+ hematopoietic stem cells (HSC) from the patient were transduced with a GALV enveloped MFGs retrovirus containing IL2RG cDNA. Transduced cells acquired new cell surface expression and functional signaling via gc in response to IL-2. Given BMT failure in this patient and recently reported XSCID gene therapy, isolation, retroviral gene correction and reinfusion of HSC offer a therapeutic approach.

Program Nr: 207 from the 2000 ASHG Annual Meeting

Dominant negative myostatin produces hypertrophy without hyperplasia in muscle. *X. Zhu[1,2], M. Hadhazy[1,2], M. Wehling[3], J.G. Tidball[3], E.M. McNally[1,2].* 1) Department of Medicine/Cardiology, University of Chicago, Chicago, IL; 2) Department of Human Genetics, University of Chicago, Chicago, IL; 3) Department of Physiological Science, University of California Los Angeles, Los Angeles, CA.

Myostatin (growth differentiation factor 8), a TGF-b family member, is a negative regulator of muscle growth. TGF-bs undergo dimerization and cleavage in the extracellular milieu to release the mature, active form derived from the carboxyl-terminus. We generated transgenic mice that express myostatin mutated at its cleavage site, therefore acting as a dominant negative ligand. Mutant myostatin was expressed under the control of the muscle specific promoter, muscle creatine kinase (MCK). The mutant ligand significantly reduced the endogenous myostatin processing up to 40%, confirming the dominant effect of the cleavage mutant. Transgenic mice expressing mutant myostatin exhibited a substantial increase (20-35%) in muscle mass that resulted from myofiber hypertrophy. This hypertrophy was seen in both fast and slow muscle fiber types. No increase in hyperplasia was noted in mice expressing dominant negative myostatin suggesting that the hypertrophic effect of myostatin was independent of its hyperplastic effect. Interestingly, this hypertrophic effect was not correlated with an increase in expression of transcription factors that have been implicated in muscle hypertrophy, including myogenin, GATA-2 and MEF-2C. This suggests that an alternative signaling pathway and/or other transcription factors mediate myostatin-inhibited hypertrophy. We also evaluated the role of endogenous myostatin in muscle degenerative states, such as muscular dystrophy, and found significant down-regulation of wild type myostatin mRNA expression. Thus, further inhibition of myostatin may permit increased muscle growth in muscle degenerative disorders. Dominant negative myostatin mice were crossed with mice lacking g-sarcoglycan to determine whether increased hypertrophy ameliorates the severe dystrophic phenotype in these mice. Percentage of central nuclei, degeneration and fibrosis were not affected by the myostatin transgene suggesting that muscle hypertrophy is insufficient to protect against the dystrophic process.

Program Nr: 208 from the 2000 ASHG Annual Meeting

A mouse model of spinal bulbar muscular atrophy (SBMA). *J.R. Morrison[1], P. McManamny[1], M.K. O'Bryan[1], K.L. Cimdins[1], I. Kola[1], S. Cheema[2], D.M. de Kretser[1].* 1) Molec Reproduction/Endocrinol, Monash Inst Reproduction/Dev,; 2) Dept Anatomy, Monash University, Clayton, Australia.

SBMA is an adult onset, X-linked neurological disorder characterized by proximal muscle wasting and androgen insensitivity. The underlying genetic mutation is an expanded trinucleotide repeat (TNR) in exon 1 of the androgen receptor (AR). Females rarely exhibited any symptoms of the disease. This is one of a group of neurological disorders associated with expanded TNR which includes several spino-cerebellar ataxias and Huntington disease. Our primary aim was to create a mouse model of SBMA. The TNR in the AR is highly polymorphic ranging from 8-31 repeats in normal individuals to more than 38 repeats in SBMA. Transgenic mice were generated carrying a full-length human AR construct with either 20, 65 or 120 TNRs. The cytomegalovirus promoter was used to drive the transgenes. Mice carrying AR-20 (10 lines), AR-65 (10 lines) and the AR-120 (2 lines) were shown to be expressing the human AR variants. Mice carrying both the AR-65 and AR-120 developed a progressive neurological phenotype which was more dramatic in the mice carrying the longer TNRs. The affected mice displayed hind-foot clenching which progressed to a full body clench in the AR-120 lines. These mice also exhibited a progressive reduction in cage activity. Both of these abnormalities have been observed in mouse models of Huntington disease. Further development in the SBMA pathophysiology resulted in severe muscle wasting which affected the rear hind limbs. The AR-120 lines develop the muscle wasting by 2 months of age while the AR-65 lines took 3-4 months to develop these symptoms. The pathology was observed in both male and female mice suggesting that, like other TNR diseases, the expanded TNR in the AR produced a gain-of-function mutation that causes the neurological phenotype. The testicular atrophy and infertility reported in humans with SBMA has not been observed in these transgenic mice suggesting that this aspect of the disease may reflect a loss-of-function.

Program Nr: 209 from the 2000 ASHG Annual Meeting

The candidate SMA phenotypic modifier SERF1 and its relative SERF2 are nucleolar gene products. *S. Lacy, J. Scharf, J. Haslett, L. Kunkel.* Dep't of Genetics, Children's Hospital, Boston, MA.

In addition to deletions and gene conversions of the SMN1 gene, the varying degrees of severity of SMA I - III have been postulated to result from the loss of an additional genetic modifying factor. The candidate SMA phenotypic modifier SERF1 (originally named H4F5) was identified as an evolutionarily conserved mouse/human gene by comparative genomics. SERF1 maps 6.5 kb upstream of exon 1 for both SMN1 and SMN2 on chromosome 5q13 and contains the C212 marker within its last intron. 94% of type I SMA patient chromosomes analyzed were deleted for one or both copies of C212, and therefore SERF1. SERF1 (and its relative SERF2) are ubiquitously expressed and show very abundant transcript levels in testis. They share 78% amino acid similarity and are predicted to be highly charged helix-turn-helix proteins due to their high content of lysine and arginine. They share limited homology to the matrix-associated RNA-binding protein matrin-cyclophilin which co-localizes with snRNPs and the cellular splicing apparatus. SERF2 maps to chromosome 15q15-21, falling within the ALS5 Type 1 critical region. We screened four ALS5 families for SERF2 mutations but none were detected. Using affinity purified SERF1 and SERF2-specific anti-peptide antibodies, we have determined that SERF1 and SERF2 are nuclear proteins in HeLa and COS-1 cells. Subsequent fractionation experiments show SERF1 and SERF2 are10-15 kDa exclusively nuclear proteins which are not associated with the nuclear matrix. Co-immunoprecipitation experiments using SMN, Sm and SC-35 antibodies suggest SERF1and SERF2 are not directly associated with these components of the spliceosome. By immunofluorescense, SERF1 and SERF2 appear in large nuclear structures which do not co-localize with the major small nuclear snRNPs (recognized by antibody Y12) or with SMN gems or with spicing factor SC-35. We have determined these nuclear structures co-localize with nucleolin in nucleoli, where they may be associated with RNA biogenesis. The nucleolar localization coupled with the deletion data make SERF1 a strong candidate as a phenotypic modifier of SMA patients but it is presently unclear exactly how this occurs.

Program Nr: 210 from the 2000 ASHG Annual Meeting

Mutant SMN protein (472del5) in Spinal Muscular Atrophy enters the nucleolus. *S. Lefebvre, S. Bertrandy, P. Burlet, C. Belser, L. Viollet, A. Munnich.* INSERM U393, Necker Institute, Hpital des Enfants-Malades, 75743 Paris cedex 15, France.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of spinal motor neurons. SMA results from mutations of the Survival Motor Neuron (SMN) gene located on chromosome 5q13. The SMN protein is markedly deficient in SMA patients. SMN is distributed in the cytoplasm and in nuclear bodies called gems, and thought to play essential roles in RNA metabolism, but how SMN functions is still poorly understood. SMN has been shown to bind proteins such as SIP1 (SMN-interacting protein 1), the Sm proteins (common spliceosomal snRNP proteins) and the fibrillarin, a nucleolar protein. Isolation of SMN orthologues allowed us to demonstrate that the RNA-binding domain was conserved across species and located in the N-terminal end of SMN. Most importantly, a deleted protein mimicking a SMA frameshift mutation showed a dramatic change in its RNA-binding properties in vitro. To characterize the human mutation, we produced E. coli recombinant proteins corresponding to the normal and mutant SMN proteins and found that the mutation altered the RNA-binding activity to ribonucleotide homopolymers. These data indicated that deletion of the C-terminal end (which binds, at least, to RNA helicase and transcription factor and includes the self-association domain) can affect the RNA-binding capacity of SMN. Therefore, the different binding properties of SMN might be closely related and serve in the assembly of ribonucleoprotein complexes. To test this hypothesis, we first demonstrated that the mutant SMN protein still binds in vitro to SIP1 and the Sm proteins. Secondly, the normal and mutant SMN proteins were overexpressed in mammalian cell cultures. The normal SMN localized in the cytoplasm and in nuclear gems, whereas the mutant protein exhibited altered nuclear distribution. Indeed, a cell-cycle dependent accumulation into the nucleolus of the mutant protein was observed, suggesting a link between SMN and the ribosome biogenesis. This cellular model should help to elucidate the function(s) of SMN and contribute to a better understand the pathogenesis of SMA.

Program Nr: 211 from the 2000 ASHG Annual Meeting

GLUT 10: A novel glucose transporter in the Type 2 diabetes linked region of chromosome 20q12-13.1. *P.A. Dawson[1], S.C. Fossey[2], S.J. Mihic[3], A.L. Craddock[1], J. Mychaleckyj[1,3,4], J.R. Snyder[2], D.W. Bowden[1,2]*. 1) Internal Medicine, Wake Forest University, Winston-Salem, NC; 2) Biochemistry; 3) Physiology & Pharmacology; 4) Public Health Sciences.

We have identified a novel human facilitative glucose transporter, designated GLUT10, that was localized to the Type 2 diabetes linked region of human chromosome 20q12-13.1, between D20S888 and D20S891. The GLUT10 cDNA encompasses 1623 bp of coding sequence, 250 bp of 5 untranslated, and 2273 bp of 3 untranslated sequence. The GLUT10 gene is encoded by 5 exons spanning 26.8 kb of genomic DNA. The GLUT10 cDNA shares between 23 and 32% amino acid identity with human GLUT1-5 and between 34 and 44% identity with plant and bacterial hexose transporters. GLUT10 has a 12 transmembrane domain predicted topology, a feature shared by all members of the hexose transporter superfamily. GLUT10 shares many sequence characteristics of mammalian glucose transporters, including the GRR/K (between TM2 and TM3) and EX6R/K (between TM4 and TM5) motifs, conserved tryptophan residues (residues 430/454) critical for glucose transport activity and two predicted N-linked glycosylation sites. Unlike GLUT1-5, GLUT10 has a 41 amino acid insertion between TM6 and TM7. Northern hybridization analysis identified a single 4.4 kb transcript for GLUT10 in heart, lung, brain, liver, skeletal muscle, pancreas, placenta, and kidney. By RT-PCR analysis, GLUT10 mRNA was also detected in ovary, prostate, salivary gland, thyroid, adrenal, small intestine, skin and fetal brain and liver (but not testes, uterus, peripheral blood lymphocytes, or bone marrow). When expressed in Xenopus oocytes, GLUT10 exhibited phloretin-inhibitable glucose transport with an apparent Km of 250 uM. Glucose uptake was also weakly competed by galactose but not fructose. Glucose transport in the GLUT10 injected oocytes was stimulated approximately 2-fold in the presence of insulin. This suggests a potential role for GLUT10, similar to GLUT4, in the insulin stimulation of enhanced glucose metabolism.

Program Nr: 212 from the 2000 ASHG Annual Meeting

Large-scale discovery of gene functions using an ink-jet oligonucleotide synthesizer and a compendium of DNA microarray expression profiles. *T.R. Hughes, P. Linsley, M. Marton, C. Roberts, A. Jones, R. Stoughton, D. Shoemaker, A. Blanchard, J. Phillips, M. Ziman, J. King, J. Burchard, E. Coffey, H. Dai, Y. He, S. Kobayashi, M. Mao, M. Meyer, J. Schelter, S. Friend.* Rosetta Inpharmatics, Kirkland, WA.

The development of DNA microarrays has been heralded as a major breakthrough for analysis of gene functions. Technical challenges in large-scale microarray-based analysis projects include the manufacture of high-quality microarrays, the management and analysis of large data matrices, and the construction of useful data sets. Here, we describe a new platform for DNA microarrays, and show how the expression profile can be used to monitor hundreds of different cellular functions simultaneously. Using an ink-jet delivery system that enables synthesis of oligonucleotides *in situ*, we have fabricated microarrays containing oligos corresponding to ~50,000 human genes on a single 9 square inch glass microscope slide. Results obtained with ink-jet arrays correlate well with those from other technologies. We have investigated the utility of a reference database or 'compendium' of microarray expression profiles corresponding to hundreds of diverse mutations and chemical treatments in *S. cerevisiae* (Hughes et al., *Cell*, in press). Our results show that perturbance of different cellular pathways causes unique and recognizable changes in the expression profile, providing a general-purpose approach to determine the functions of novel genes. We are now engaged in the challenge of applying ink-jet microarrays and compendia of expression data to discover and characterize gene functions in *Homo sapiens*.

Program Nr: 213 from the 2000 ASHG Annual Meeting

Statistical methods for human nondisjunction data. *J. Li[1], H. Zhao[1], S.L. Sherman[2], W.P. Robinson[3].* 1) Epidemiology and Public Health, Yale University Medical School, New Haven, CT; 2) Emory University School of Medicine, Atlanta, GA; 3) University of British Columbia, Vancouver.

Nondisjunction refers to the presence of two copies of a chromosome in a gamete. Chromosomal nondisjunction may lead to uniparental disomy (UPD) and trisomy. For UPD individuals, the chromosome number is normal but both homologs of a chromosome pair have originated from a single parent. UPD is associated with Prader-Willi syndrome and Angelman syndrome. Trisomy is the leading cause of mental retardation, and is the result of one parent transmitting a disomic gamete, and the other parent transmitting the usual monosomic gamete. Genetic studies of UPD and trisomy employing many markers have helped geneticists to gain a better understanding of the molecular mechanisms underlying nondisjunction. However, the valuable information from such studies has not been fully utilized by existing methods. We have developed a general approach to analyzing nondisjunction data that can simultaneously handle multiple markers (including missing and uninformative markers) and also can incorporate crossover interference. Under the assumption that there is at most one crossover within each marker interval, we will discuss how to use our approach to recover exchange patterns during meiosis. In addition, we will present a hidden Markov model (HMM) for nondisjunction data. The major advantage of this HMM model is that the amount of computation increases linearly with the number of markers analyzed. We have implemented our methods in computer programs and carried out extensive simulations to study the performance of our methods. We will summarize our simulation results and describe the applications of our methods to UPD 15 data and trisomy 21 data.

Program Nr: 214 from the 2000 ASHG Annual Meeting

Meta analysis of relative penetrance rank order statistics with application to HLA DR-DQ genes and type 1 diabetes. *S. McWeeney, G. Thomson.* Integrative Biology, UC Berkeley, Berkeley, CA.

The direct involvement of the HLA DR-DQ genes (DRB1, DQA1, and DQB1) in type 1 diabetes is well established. These genes display a complex hierarchy of predisposing, intermediate, and protective effects at the genotype and haplotype levels. The ratio of the observed frequency of a genotype (haplotype) in patients over the frequency in controls, referred to as the P/C (patient/control) ratio, is an MLE of the relative genotype (haplotype) penetrance values. A novel test has been developed to compare the relative rank orders of predisposing through protective P/C ratios of genotypes and haplotypes across ethnic groups. The algorithm developed to determine the probability of an observed rank order in a population being that close by chance to a putatively "known" rank order, allows for the fact that not all genotypes (haplotypes) will be found in every population. The key to development of the algorithm is use of a recurrence relationship to determine the probabilities when an additional genotype (haplotype) is added to the analysis. Meta analysis of the resulting p values across populations is weighted by sample size. Consistency in rank order of P/C ratios of HLA DR-DQ genotypes and haplotypes in type 1 diabetes is seen across ethnic groups. This allows investigation of the specific amino acids at the HLA DR-DQ genes involved in type 1 diabetes. The rank order method developed is applicable to other genetic regions besides HLA.

Program Nr: 215 from the 2000 ASHG Annual Meeting

A cellular automata-based pattern recognition approach to identifying gene-gene and gene-environment interactions. *J.H. Moore, L.W. Hahn.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

It is increasingly clear that gene-gene and gene-environment interactions play an important role in determining risk of common complex diseases with multifactorial etiologies. We have developed an approach to identifying gene-gene and gene-environment interactions in discordant sib-pair study designs that combines the power of cellular automata (CA) for pattern recognition and genetic algorithms for machine learning.

CAs are dynamic systems that consist of an array of cells, each with a finite number of states that are updated at discrete time steps according to specific rules. The state of a cell at the next time step is determined by the current states of the neighboring cells. CAs can be used to perform computations by taking advantage of features such as massive parallelism. We have adapted a CA to accept an array of genotypes and/or environmental classes as input and produce an array of information as output that can be used to classify sibs as affected or unaffected (e.g. f(010110|affected) = 111111 or f(100010|unaffected) = 000000). In addition, we have adapted the genetic algorithm machine learning methodology and cross-validation to identify combinations of genotypes and/or environmental classes and CA parameters necessary to correctly classify affected and unaffected sibs.

Using simulated data, we have demonstrated that this approach can identify high-order interactions in small sample sizes in the absence of marginal or main effects. The results of this study suggest that pattern recognition approaches will be useful for the identification of genes that influence susceptibility to common complex diseases only through their interaction with other genetic and/or environmental factors.

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Program Nr: 216 from the 2000 ASHG Annual Meeting

Combinatorial partitioning reveals interactive effects of the *ACE I/D* and *PAI-1 4G/5G* polymorphisms on plasma PAI-1 levels. *J.M. Lamb, N.J. Brown, M.T. Baker, J.V. Gainer, K.R. Richardson, D. Connolly, A. Gupta, D.E. Vaughan, J.H. Moore.* Program in Human Genetics, Vanderbilt University, Nashville, TN.

There is accumulating evidence that plasma levels of plasminogen activator inhibitor 1 (PAI-1), a risk factor for thrombosis, may be influenced by biological interactions between the renin-angiotensin and fibrinolytic systems. The goal of this study was to determine whether there is statistical evidence for interactive effects of genes from these two biochemical systems on plasma PAI-1 levels in a sample of 49 African Americans and 106 Caucasians.

Plasma PAI-1 antigen levels were measured using an enzyme-linked immunosorbant assay. We measured the *insertion/deletion (I/D)* polymorphism in the *angiotensin converting enzyme (ACE)* gene and the *4G/5G* polymorphism in the *PAI-1* gene. Because analysis of variance (ANOVA) has limited power to detect gene-gene interactions in small sample sizes, we utilized the combinatorial partitioning method (CPM) of Nelson et al. (Genome Research, in press) to test for interactive effects of the *ACE I/D* and *PAI-1 4G/5G* polymorphisms on plasma PAI-1 levels. CPM is a data reduction technique that pools multi-locus genotypes into equivalent classes thus gaining back degrees of freedom lost when using standard ANOVA.

Using CPM, we found statistically significant evidence for an interaction between the *ACE* and *PAI-1* genes in both African American females ($R^2=0.223$, $P<0.05$) and males ($R^2=0.217$, $P<0.05$) and Caucasian females ($R^2=0.102$, $P<0.05$) and males ($R^2=0.107$, $P<0.05$). These interactions were not detected when standard ANOVA was used. This study illustrates the importance of considering interactions between the renin-angiotensin and fibrinolytic systems for understanding the genetic architecture of plasma PAI-1 levels.

Program Nr: 217 from the 2000 ASHG Annual Meeting

Apo(a) gene variation and Lp(a) levels in 15 world populations towards the global picture. *G. Utermann[1], M. Scholz[2], R. Delport[1], C. Ehnholm[3], F.S. Geethanjali[1], K. Luthra[1], F. Kronenberg[1], L. Lemming[1], A. Lingenhel[1], O. Makemaharn[1], R.W.C. Pang[1], K. Schmidt[1], A. Abe[1], M. Volkova[1], M. Windegger[1], M. Ogorelkova[1], H. Bickeboeller[2], H.G. Kraft[1]*. 1) Inst Medical Biol & Human Gen, Innsbruck, Tyrol, Austria; 2) Med.Statistics and Epidemiology, Munich, Germany; 3) National Public Health Institute, Helsinki, Finland.

The apo(a) gene codes for a large glycoprotein which together with LDL forms the Lp(a) complex which is associated with atherothrombotic disease and represents a quantitative genetic trait in human plasma. Human Apo(a) contains a protease domain, ten different plasminogen related kringle IV-domains (K IV-1-10) and one K V domain. Unique to the apo(a) gene is a variable number (n = 2-43) of identical 5.6 kb repeats containing exons coding for the kringle IV-type 2 domains in apo(a) (= K IV-2 VNTR). Lp(a) concentrations, the K IV-2 VNTR, a pentanucleotide repeat polymorphism and two SNPs, one in the 5and one in the 3regions of the apo(a) gene were analyzed in > 2000 unrelated subjects from 15 populations of African, Arctic, Asian, European, and Pacific origins. Considering median apo(a) K IV-2 repeat number and Lp(a) levels African, Asian, and European populations formed distinct clusters characterized. Africans had the highest Lp(a) and lowest K IV-2 repeats and Caucasians the lowest Lp(a) and highest K IV-2 repeats. Haplotype frequencies were also strikingly different between populations of different geographic origins. Significant linkage disequilibria exist between close polymorphisms in the 5flanking region and between SNPs separated by the K IV-2 repeats. They are conserved across populations suggesting that the K IV-2 VNTR has evolved by gene conversion rather than by recombination. A systematic analysis of SNPs in apo(a) exons coding for K IV-6-10 was performed in a subset of populations (n > 300). Thereby some population-restricted "young" SNPs were found associated with a wide range of K IV-2 repeat alleles and some did affect Lp(a) levels. Together the data suggest that the genetic system maintaining high Lp(a) levels in Africa deteriorated when modern humans migrated out of Africa. ‘’>’>”“.

Program Nr: 218 from the 2000 ASHG Annual Meeting

Resolution of linkage heterogeneity in complex traits: regression tree linkage analysis of blood pressure in the NHLBI-HyperGEN Study. *M.A. Province[1], S.C. Hunt[2], W. Shannon[1], R.H. Myers[3], D.K. Arnett[4], J. Pankow[5], C.E. Lewis[5], D.C. Rao[1]*. 1) Dept Biostatistics, Washington Univ Sch Medicine, St Louis, MO; 2) Univ Utah, Salt Lake City, UT; 3) Boston Univ, Boston, MA; 4) Univ Minnesota, Minneapolis, MN; 5) Univ North Carolina, Chapel Hill, NC.; or Univ Alabama, Birmingham, AL.

For complex traits like hypertension (HT), different risk genes may each work through unique context dependencies (e.g. obesity, impaired renal function, renin-angiotensin system activation, insulin resistance, etc.). Any HT sibship sample is likely a heterogeneous mix, which hinders gene finding, since a given locus may be important in only a fraction of the sibships. One promising approach is regression tree linkage (Shannon et al. 2000; Province et al., 2000) which recursively partitions the sibships into relatively more homogeneous linkage subsets for a locus via optimally splitting by their measured covariates. Unlike standard classification/regression tree models (which cluster on the phenotype or measured genotype in individuals only), tree linkage partitions sibships directly on the linkage evidence. This works well in simulation, boosting the overall linkage power from 29% to 78% for a two-locus trait in a low signal-to-noise sample where only 1/5 of all sibpairs are informative for either locus. In 231 African-American HT sibpairs genotyped in the multicenter HyperGEN study, systolic blood pressure was only weakly linked overall (Haseman-Elston (H-E) slope b = -0.64 P=0.14) to a promising marker on Chrom 2 (GATA11H10 38 cM p-ter). Tree linkage identified two subsets: a linked set of N=120 sibpairs defined by having one or more combinations of low BMI, low triglyceride and/or low urine sodium values (H-E b=-1.67 P=0.02); and the remaining unlinked N=111 sibpairs (H-E b=0.53 P=0.77), suggesting that a nearby gene may be acting in the context of a low HT risk profile. Validation of these results and others await further sibpair genotyping. This tool may more sensitively localize complex trait genes in the face of etiologic heterogeneity, and identify meaningful combinations of context dependencies governing gene expression.

Program Nr: 219 from the 2000 ASHG Annual Meeting

Genetic Modification of the Beneficial Effect of Alcohol Consumption on HDL Levels and Coronary Heart Disease. *L.M. Hines[1], M.J. Stampfer[1,2,3], J. Ma[2], J.M. Gaziano[4,5], P.M. Ridker[4], S.E. Hankinson[1,2], F. Sacks[2,3], E.B. Rimm[1,2,3], D.J. Hunter[1,2,3]*. 1) Dept of Epidemiology, Harvard School of Public Health, Boston, MA; 2) Channing Laboratory, Dept of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, MA; 3) Dept of Nutrition, Harvard School of Public Health, Boston, MA; 4) Divisions of Preventative Medicine and Cardiology, Harvard Medical School, Boston, MA; 5) MA Veterans Epidemiologic Research and Information Center, Veterans Administration, Boston, MA.

Moderate alcohol consumption is associated with reduced risk of myocardial infarction (MI) in epidemiologic studies. However, some have suggested that moderate alcohol consumption may be a surrogate for favorable socioeconomic or lifestyle factors. Further, the benefit of alcohol consumption has been attributed to constituents in alcoholic beverages other than ethanol. A polymorphism in *ADH3* has been shown to alter the rate of ethanol metabolism, and may mediate the effect of alcohol on susceptibility to MI.

We investigated the relationship between *ADH3* genotype, alcohol consumption, and risk of MI in a case-control study (396 cases and 770 controls) nested within the prospective Physicians' Health Study cohort. We observed a marked and significant interaction (P = 0.01). Compared to men who consumed less than 1 drink/week and were homozygous for the fast oxidizing allele, there was a highly significant 86% reduction in risk of MI for daily drinkers who were homozygous for the slow oxidizing allele (multivariate RR = 0.14; 95% CI = 0.04-0.45).

In addition, we observed an interaction between *ADH3* genotype and alcohol consumption on plasma HDL levels (P, interaction = 0.046). HDL levels were highest for the slow oxidizers, followed by the intermediate and fast oxidizers. A similar relation was observed among 325 postmenopausal women (not taking hormone replacement therapy) in the Nurses' Health Study (P, interaction = 0.02). This study provides strong evidence that ethanol is responsible for the protective effect of alcohol consumption on risk of MI.

Program Nr: 220 from the 2000 ASHG Annual Meeting

Interaction of chromosome 10q and 20q loci influence extreme human obesity. *R.A. Price.* Center for Neurobiology and Behavior, Univ Pennsylvania, Philadelphia, PA.

Gene interactions are likely to play an important role in determining complex phenotypes. Human obesity is a multigenic trait previously linked to several chromosome regions. The current study screened for possible genetic interactions by correlating the proportions of alleles shared identical by descent (IBD) for extremely obese (body mass index > 35) sibling pairs at unlinked loci in each of three previously identified linked regions on chromosomes 7q, 10q and 20q. Sibling pair IBD correlations for chromosome 7 markers and IBD in the other two regions approached zero, indicating no interaction with chromosome 7 loci. However, there were positive correlations of IBD sharing for several loci in chromosomes 20q with markers in 10q. The correlation reached a maximum of 0.30 (273 pairs, $p<0.0000002$) between markers corresponding to two previous single-locus linkage peaks in 10q and 20q. The correlation was significant after correction for multiple tests. European- and African-American subsamples separately reached nominal significance, with the latter maintained after correction for multiple tests, providing independent replication. Principal components analyses also revealed a correlation in IBD between axes representing the two chromosome regions. These results strongly suggest gene interactions between loci in these regions influence extreme human obesity. Supported in part by NIH Grants R01DK44073, R01DK48095, and R01DK56210 to RAP.

Program Nr: 221 from the 2000 ASHG Annual Meeting

A promoter allele in the eotaxin chemokine gene protects against HIV-1 infection. *W.S. Modi[1], J.J Goedert[2], T.R. O'Brien[2], S. Buchbinder[3], J. Giorgi[4], C. Rinaldo[5], S. Donfield[2], S.J. O'Brien[2].* 1) SAIC, NCI-Frederick Cancer Res., Frederick, MD; 2) National Cancer Institute, Frederick/Rockville, MD 21702/20852; 3) San Francisco Department of Public Health, San Francisco, CA; 4) Department of Medicine/CIC, UCLA School of Medicine, Los Angeles, CA 90095-1745; 5) Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261.

Allelic variation in various host genes has been shown to bear on AIDS pathogenesis. A novel allele called G-1328A in the promoter region of the eotaxin gene modifies an activator protein-2 transcription factor binding site. Since HIV-1 is known to use the eotaxin receptor CCR3 to infect cells of the central nervous system, association analyses in cohorts were carried out to test whether this allele has any effect on infection and/or disease progression. Results indicate that the frequencies of genotypes containing G-1328A (the heterozygotes and mutant homozygotes) are significantly higher in exposed uninfected patients than in seroconvertors. This protective effect was found among both homosexuals and hemophiliacs. For example, among all samples the frequency of a protective genotype in 524 seroconverters is 32.2%, whereas among 154 high-risk exposed uninfected patients it is 45.5% (odds ratio=0.571, $p<0.003$). Considering the MACS alone, the frequency of a protective genotype among 333 seroconverters is 30.6%, while among 77 high-risk exposed uninfected samples it is 44.2% (odds ratio=0.558, $p<0.03$). Results from Cox regression analyses were not significant for any of the disease progression end points monitored. Allelic variation in the eotaxin gene may protect against HIV-1 infection by: a) limiting the availability of CCR3, or b) mediating an effective anti-HIV-1 cytotoxic T-lymphocyte response. This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-56000.

Program Nr: 222 from the 2000 ASHG Annual Meeting

The imprinting TDT and missing parental genotype reconstruction: the IRC-TDT. *S. Horvath[1,2]*. 1) Dept. Biostatistics, Harvard School of Public Health, Boston; 2) IMBIE, University of Bonn.

Family based allelic association tests are useful for addressing 2 different question about imprinting: first, if linkage disequilibrium (LD) has been detected the investigator may wish to look further for imprinting effects; second, if LD has not yet been detected the investigator may wish to consider imprinting effects to increase the statistical power. For the first question, Weinberg (1999, Am J Hum Genet 65:229-235) proposes methods involving family trios. For the second question, a straightforward solution exists for the case of known genotypes: stratify the transmission/non-transmission counts of the TDT (Spielman and Ewens 1993; Am J Hum Genet Suppl 53:863) according to whether the mother or the father is the source. But this imprinting TDT is in general not applicable when one (or both) parents are missing. Here, I generalize this test to the case of missing parental genotypes by using an idea based on the reconstruction combined TDT introduced by Knapp (1999, Am J Hum Genet 64:861-870): reconstruct the missing parental genotypes from offspring genotypes when possible, count the number of times a heterozygous mother (father) transmits the candidate allele to her affected offspring, correct for the bias introduced in the reconstruction step by conditioning on reconstructibility. The resulting imprinting RC-TDT (IRC-TDT) is a non-parametric test that makes no assumption about the mode of inheritance, the ascertainment of the sample and it protects against population admixture effects. Even if no parental genotypes are available, one can gain additional information, by using the dominant - instead of the standard additive marker coding RC-TDT. I use simulation studies to discuss the power of the test but also report the results of applying the method to type 1 diabetes (IDDM) data involving the insulin gene (INS, IDDM2). The IRC-TDT is implemented in a freely available SAS program, which can calculate exact P values.

Program Nr: 223 from the 2000 ASHG Annual Meeting

Effect of Hierarchical Clustered Sampling in Multicenter, Family-based Studies: Example of Reproductive History and Breast Cancer Risk in BRCA1 Mutation Carriers. *Y. Wang[1], T. TenHave[1], H.T. Lynch[2], J-S. Brunet[3], S.A. Narod[3], J.E. Garber[4], A.K. Godwin[5], M.B. Daly[5], T.R. Rebbeck[1]*. 1) University of Pennsylvania, Philadelphia, PA; 2) Creighton University, Omaha, NE; 3) Women's College Hospital, Toronto, ON; 4) Dana Farber Cancer Institute, Boston, MA; 5) Fox Chase Cancer Center, Philadelphia, PA.

Studies that involve high penetrance genes (e.g., BRCA1 and breast cancer or CDKN2a and melanoma) are often undertaken using multicenter, family-based study designs. These hierarchical sampling designs arise by necessity because the frequency of disease-associated mutations in these genes in the general population is rare. A result of this sampling design is non-independence of observations and unbalanced samples that may result in biases to both the point estimates and confidence intervals computed using standard analytical methods. We propose a set of nonlinear models with a focus on the Cox model that account for the potential effects of confounding by family or center, and apply a robust variance estimation approach to correct for the effect of non-independence of observations. We have applied these methods to evaluate the role of reproductive factors in modulating breast cancer risk in a sample of 281 women who carry germline mutations in BRCA1 drawn from 111 families at five referral centers. The results of Cox proportional hazards models estimating hazard ratios (HR) suggest that age at menarche and age at first live birth play little or no role in modifying breast cancer risk among women with BRCA1 mutations. High parity (>2 live births) conferred a significant breast cancer risk reduction (HR=0.45, 95%CI: 0.32-0.64). The effect of parity was confounded by family but not by ascertainment center. Adjustments for this confounding altered the inferences of these analyses by reducing the protective effect of parity on breast cancer risk (HR=0.61, 95%CI: 0.37-0.99). These results suggest that samples drawn in a hierarchical clustered manner may require specific analytical consideration in order to obtain unbiased point estimates and confidence intervals.

Program Nr: 224 from the 2000 ASHG Annual Meeting

Testing the Hypothesis that Assortative Mating has Increased the Frequency of Cx 26 Deafness. *W.E. Nance[1], X-Z. Liu[1], R. Erdenetungalag[2], J. Radnaabazar[2], B. Dangaasuren[2], A. Pandya[1]*. 1) Dept Human Genetics, Virginia Commonwealth Univ, Richmond, VA; 2) Dept Maternal and Child Health, Ulaanbaatar, Mongolia.

Using a novel extension of segregation analysis, we have shown that the frequency of homozygosity for Connexin 26 (Cx 26) mutations among the deaf in the U.S has increased from about 19% to 35% since 1800. We proposed the increasing frequency may be caused in part by the intense assortative mating for deafness in this country during that period (Lancet, in press). For a genetically heterogeneous recessive trait such as deafness, assortative mating preferentially increases the frequency of the commonest recessive genotype since these individuals account for a disproportionate share of the non-complementary matings that can only produce deaf offspring. To test our hypothesis, we screened for Cx 26 mutations in a sample of deaf Mongolian students who belong to a population without a long tradition of intermarriages among the deaf. At least half of the Mongolian population still lead a nomadic lifestyle; deaf students have been educated at a central school in Ulaanbaatar only since the mid 1950's; sign language was not introduced until 1994 and among 620 deaf probands at the school none had two deaf parents. Cx 26 testing has been completed by direct fluorescent sequencing of the coding exon in probands from 10 simplex and 18 multiplex sibships. Eleven were found to carry the V27I polymorphism, which has also been noted in other populations but no examples of the 35DelG mutation or any other pathologic allele was encountered. In contrast, the expected number of Cx 26(+) cases in a comparable U.S. sample would be 8($p<0.005$). The low frequency of Cx 26(+) cases in a population where deaf marriages have been very uncommon in the past supports the view that assortative mating may explain the high frequency of this trait in other populations. Low Cx 26(+) rates in Japan and anecdotal reports from China and India are consistent with our data. If our hypothesis is correct, assortative mating may represent another important mechanism in addition to genomic instability and heterozygote advantage which can maintain specific phenotypes at unexpectedly high frequencies in the population.

Program Nr: 225 from the 2000 ASHG Annual Meeting

Generation of Mouse Models for Friedreich Ataxia. *H. Puccio[1], D. Simon[1], M. Cosse[1], A. Gansmuller[1], L. Reutenauer[1], R. Matyas[1], P. Rustin[2], J.L. Mandel[1], M. Koenig[1]*. 1) IGBMC, INSERM, CNRS, ULP- Strasbourg, France; 2) Hôpital Necker Enfants Malades, Paris, France.

Friedreich ataxia (FRDA), the most common autosomal recessive ataxia, is due to a partial loss of frataxin, a mitochondrial protein conserved through evolution, and involved in mitochondrial iron homeostasis. Yeast knock-out models, and histological and biochemical data from patient heart biopsies or autopsies indicate that the frataxin defect causes a specific iron-sulfur protein deficiency and mitochondrial iron accumulation leading to the pathological changes. To study the mechanism of the disease, we are generating mouse models by deletion of exon 4 leading to inactivation of the Frda gene product. Homozygous deletions cause embryonic lethality a few days after implantation with the absence of iron accumulation. These results demonstrate an important role for frataxin during early development but do not reproduce any characteristics of the human disease.

To circumvent embryonic lethality, we have generated two different conditional knock-out models, based on the Cre-lox system, in which exon 4 has been deleted either specifically in skeletal and cardiac muscle (MCK) (using a transgenic mice expressing Cre under the Muscle Creatinine Kinase promoter- gift from Dr. Kahn, Boston) or predominantly in neuronal tissues (NSE) (Neuron Specific Enolase promoter; gift from Dr. Melki, France). We have obtained viable animals for both models that reproduce some morphological and biochemical features observed in FRDA patients. MCK animals develop cardiac hypertrophy, cardiac specific iron-sulfur protein deficiency and die at 10 weeks of age. NSE animals develop a progressive loss of proprioception, ataxia, dilated cardiomyopathy, cardiac specific iron-sulfur protein deficiency and die at 12-36 days of age. In addition to the biochemical profile typical of FRDA disease, this model reproduces some of the neurological dysfunction and neuropathological findings observed in patients. These two models will allow us to investigate, if any, the course of iron and iron-sulfur cluster homeostasis disturbances and to test anti-oxidant therapies.

Program Nr: 226 from the 2000 ASHG Annual Meeting

The role of the interaction of Huntingtin with p53 and GAPDH in the pathology of Huntington's disease. *N. Agrawal[1], M. Fujimoro[2], S. Igarashi[3], P.H. Hwang[2], T. Taya[4], M. Takahashi[1], C.D. Ferris[1], X. Luo[1], M. Fang[1], S. Sutcliffe[1], S.D. Hayward[2], C.A. Ross[3], S.H. Snyder[1], A. Sawa[1].* 1) Neuroscience; 2) Oncology Center; 3) Psychiatry, Johns Hopkins University, Baltimore, MD; 4) National Cancer Center Research Institute, Tokyo, Japan.

Mutant Huntingtin (Htt) plays a central role in the pathogenesis of HD causing cellular dysfunction and cell death. An approach to study the mechanism by which Htt leads to cellular dysfunction and cell death is to characterize proteins associated with Htt. Recently p53, a transcription factor, has been reported to interact with Htt directly. We have evaluated p53 accumulation after the expression of Htt. In differentiated PC12 cells stably expressing an inducible Htt construct, and in transient transfection of Htt constructs into N2a cells, HEK293 cells, and Hela cells, we observed an accumulation of p53 in the nuclear fraction only upon mutant Htt, but not normal Htt. The accumulation occurs prior to robust cell death. Alternatively, Htt has also been reported to interact with GAPDH. We screened for GAPDH interacting proteins using the yeast two-hybrid system and identified a novel interaction with Siah, a rat clone homologous to the Drosophila Sina. GAPDH in the nucleus stabilized Siah, which was confirmed by pulse chase experiments, and augment its toxicity. Siah can facilitate degradation of N-CoR and other nuclear co-repressors culminating in alteration of gene expression. Consequently, we are now analyzing the precise mechanism of transcriptional modulation that mediates cellular dysfunction and cell death in HD.

Program Nr: 227 from the 2000 ASHG Annual Meeting

Methylation of FSH muscular dystrophy-associated DNA repeats. *M. Ehrlich[1], F. Tsien[1], D. Figlewicz[2], S. Winokur[3]*. 1) Program in Human Genetics, SL31, Tulane Medical Sch., New Orleans, LA; 2) Dept. of Neurology, Univ. Rochester Medical Center, Rochester, NY; 3) Dept. of Biol. Chemistry, Univ. Calif., Irvine, CA.

Deletions of the 3.3-kb subtelomeric 4q35 DNA repeat are responsible for FSHD (facioscapulohumeral muscular dystrophy), an autosomal dominant disease of unusual molecular etiology. FSHD patients have 1 - 10 copies of this repeat (D4Z4) on one of their chromosome 4 homologues instead of 11 - 150 copies on both homologues as in normal individuals. These repeats are part of a family of related sequences present in various locations in the human genome, including the subtelomeric region of chromosome 10 (10q26) although only deletions of the 4q35 repeats are associated with the disease. By Southern blotting, we showed that the D4Z4 repeat is highly methylated at Eag I sites specifically within the 4q35 region as well as at other chromosomal locations in a variety of postnatal somatic tissues, normal lymphoblastoid cell lines (LCLs), and FSHD LCLs. FSHD and normal myoblast cultures are under investigation. We found that D4Z4 repeats are also highly methylated at Sma I sites in the above-mentioned tissues and LCLs. However, this repeat is hypomethylated in normal human sperm, as are many DNA sequences that we previously characterized as exhibiting sperm-specific DNA hypomethylation. Surprisingly, D4Z4 repeats at 4q35 are also consistently hypomethylated in LCLs from patients with ICF (immunodeficiency, centromeric region instability, facial anomalies), a recessive syndrome associated with a partial DNA methylation-deficiency. Methylation of D4Z4 repeats in normal and FSHD somatic cells is consistent with the hypothesis that this region in 4q35 is generally heterochromatic because constitutive heterochromatin repeats are often highly methylated in vertebrate DNA. The presence of many methylated D4Z4 repeats at 4q35 may be required for silencing some genes in the vicinity of the D4Z4 repeats that are abnormally expressed in FSHD as a consequence of the disease-related low copy-number of these repeats. Supported in part by NIH Grant 1R01CA81506.

Program Nr: 228 from the 2000 ASHG Annual Meeting

Mutational analysis of *MECP2* in classic Rett syndrome: an update and analysis of expression of truncating alleles. *I.B. Van den Veyver[1], R.E. Amir[1], R. Schultz[1], C.Q. Tran[1], D.G. Glaze[1], H.Y. Zoghbi[1,2]*. 1) Baylor Col Medicine, Houston, TX; 2) Howard Hughes Med Inst, Houston, TX.

We established that mutations in *MECP2* (Xq28), encoding methyl-CpG binding protein 2, cause the X-linked dominant neurodevelopmental disorder Rett syndrome (RTT). MeCP2 binds to methylated cytosines in CpG islands, resulting in transcriptional repression of downstream genes. *MECP2* undergoes X-chromosome inactivation (XCI) and we reported skewing of XCI in patients with a less severe phenotype, in asymptomatic carrier females, and in classic RTT in patients with early truncations in the methyl-CpG binding domain (MBD), while over 90% of classic RTT patients have random XCI patterns. (Amir et al, Ann Neurol 2000;47:670-9). To date, we have identified 75 mutations in 92 sporadic patients (85%) and in 4 of 9 familial cases. We found 30 missense (which cluster in the methyl-CpG binding domain of MeCP2), 35 nonsense, 13 frameshift and 1 splice site mutation. Forty-six of 48 truncating mutations are beyond the MBD, 44 of which affect the transcription repression domain (TRD). The type of mutation does not correlate with overall clinical severity. XCI appears to be the major determinant of phenotypic variability. The *MECP2* gene sequence is highly conserved in a 5'UTR exon and in the 8.5 Kb 3'UTR. We therefore initiated screening of the untranslated regions in patients who do not have coding region mutations. So far, we did not find mutations in 16 such patients by screening 45% of the 3'UTR. Based on the mutation spectrum, we hypothesize that the majority of RTT-causing mutations in *MECP2* generate a peptide that retains the MBD. To test this hypothesis, we analyzed lymphoblast-derived RNA by RT-PCR followed by restriction enzyme digestion and found that the *MECP2* allele is expressed in four patients who have nonsense mutations and were informative for this assay. This suggests that the mutant protein is being produced. In summary, mutations in *MECP2* account for the majority of classic RTT. Most mutations result in a protein with an altered amino acid or one that leaves the MBD intact, suggesting that partial MeCP2 activity may be retained.

Program Nr: 229 from the 2000 ASHG Annual Meeting

A novel nemaline myopathy in the Amish caused by a mutation in troponin T1. *J.J. Johnston[1], R.I. Kelley[2, 3], T.O. Crawford[2], D.H. Morton[3], R. Agarwala[4], T. Koch[5], A.A. Schäffer[4], C.A. Francomano[1], L.G. Biesecker[1]*. 1) National Human Genome Research Institute, NIH, Bethesda, MD; 2) Johns Hopkins University, Baltimore, MD; 3) The Clinic for Special Children, Strasburg, Pa; 4) National Center for Biotechnology Information, NIH, Bethesda, MD; 5) Konrad-Zuse-Zentrum für Informationstechnik, Berlin, Germany.

The nemaline myopathies are characterized by weakness and eosinophilic, rod-like (nemaline) inclusions in muscle fibers. Amish nemaline myopathy is a distinct form of nemaline myopathy common among the Old Order Amish. In the first months of life, affected infants have tremors with hypotonia and mild contractures of the shoulders and hips. Progressive worsening of the proximal contractures, weakness and a pectus carinatum deformity develop before the children die of respiratory insufficiency, usually in the second year. The disorder has an incidence of approximately 1 in 500 among the Amish and it is inherited in an autosomal recessive pattern. Using a genealogy database, automated pedigree software, and linkage analysis of DNA samples from four sibships, we identified a 5 cM interval on chromosome 19q13.4 that was homozygous in all affected individuals. The gene for the sarcomeric thin-filament protein, slow skeletal muscle troponin T (*TNNT1*), maps to this interval and was sequenced. We identified a stop codon in exon 11, predicted to truncate the protein at amino acid 179, which segregates with the disease. We conclude that Amish nemaline myopathy is a distinct, heritable, myopathic disorder caused by a mutation in *TNNT1*.

Program Nr: 230 from the 2000 ASHG Annual Meeting

Genetic mapping of a modifier gene that delays ALS onset in a transgenic mouse model. *C.B. Kunst[1], L. Messer[1], J. Gordon[2], J.L. Haines[3], D. Patterson[1]*. 1) Eleanor Roosevelt Inst, Denver, CO; 2) Mount Sinai School of Medicine, New York, NY; 3) Vanderbilt University Medical Center, Nashville, TN.

Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by the progressive degeneration of motor neurons in the spinal cord, brain stem and pre- and postcentral neocortical gyri. Familial cases account for 10-25% of all ALS with the majority of cases being sporadic. The genetic etiology of ALS is heterogeneous and includes dominant, recessive and X-linked forms. Mutations in the cytoplasmic Cu/Zn superoxide dismutase (SOD1) gene on human chromosome 21q22.1 cause 10-20% of the familial ALS cases. The expression of the ALS phenotype in mice carrying the murine G86R SOD1 mutation is highly dependent upon the mouse genetic background. In the FVB/N background, mice expressing mG86R SOD1 develop an ALS phenotype at ~100 days with a range from 90 to 120 days. However, when these mice were bred into a mixed background of C57Bl6/129Sv the onset of the ALS phenotype was delayed (143 days to >2 years). Using 105 polymorphic autosomal markers in a whole genome scan, at ~15 cM intervals, we have identified a major genetic modifier locus with a maximum LOD score of 4.59 on mouse chromosome 13 between D13mit36 and D13mit76. This 5-8 cM interval contains the spinal muscular atrophy associated gene Smn (survival motor neuron) and 7 copies of the Naip (neuronal apoptosis inhibitory protein) gene. The identification of the gene(s) that suppress the development of ALS will have important consequences. If we can identify the gene(s) that reduce the penetrance of this mutation, we should be able to elucidate the mechanisms by which mutations in SOD1 lead to ALS. If the functional changes induced by these genetic differences can be mimicked by pharmaceutical therapeutics, the treatment of ALS and perhaps other neurodegenerative disorders in people may be possible.

Program Nr: 231 from the 2000 ASHG Annual Meeting

Mutations of a new WD-repeat protein gene in Allgrove (Triple A) syndrome. *A. TULLIO-PELET[1], R. SALOMON[1], S. HADJ-RABIA[1], C. MUGNIER[1], M.H. de LAET[2], P. BROTTIER[3], B. CHAOUACHI[4], F. BAKIRI[5], J.L. CHAUSSAIN[1], J. WEISSENBACH[3], A. MUNNICH[1], S. LYONNET[1]*. 1) INSERM U-393 et Departement de Genetique, Hopital Necker-Enfants Malades, Paris, France; 2) Service de Chirurgie Pediatrique, Hopital d'Enfants, Bruxelles, Belgium; 3) CNS, Evry, France; 4) Service de Chirurgie Pediatrique B, Hopital d'Enfants, Tunis, Tunisia; 5) Service d'Endocrinologie, Hopital Bologhine, Alger, Algeria.

Allgrove (Triple A) syndrome (MIM231550) is an autosomal recessive disorder characterized by ACTH-resistant adrenal insufficiency, achalasia of the oesophageal cardia, alacrimia and progressive neuroderenerative symptoms. While conventional linkage and homozygosity mapping allowed us to reduce the Triple a gene interval to 3.9 cM only, strong linkage disequilibrium in North African families further reduced this critical region to 0 cM as all African patients shared a rare haplotype. We sequenced a 180kb BAC encompassing the triple A minimal region and identified a novel gene encoding a 547-aminoacid protein that is mutated in affected individuals. A total of five homozygous mutations were detected in 14 patients, each of which resulted in either a premature stop codon or a frameshift, predicted to produce a truncated presumably nonfonctional protein. The founder effect in North Africa was ascribed to a single splice-donor site mutation that occurred more than 2,400 years ago. Since the candidate gene is strongly expressed in organs and tissues targeted in the disease such as neuroendocrine and cerebral structures, we believe that it is the disease causing gene now referred as ADRACALA. The predicted gene product belongs to the WD-repeat family of regulatory proteins suggesting that a novel disease mechanism is involved in the Triple A syndrome. CSA, the protein involved in Cokayne syndrome, also belongs to the WD-repeat protein family and both disorders share progressive neurodegenerative features including cerebellar ataxia and neuropathy. Hopefully, the elucidation of the triple A gene function will shed light on the development of autonomous functions and on other disorders causing progressive involvement of the central and peripheral nervous system.

Program Nr: 232 from the 2000 ASHG Annual Meeting

Large Expansion of ATTCT Pentanucleotide Repeat in Spinocerebellar Ataxia Type 10 (SCA10). *T. Matsuura[1], D.L. Burgess[1], T. Yamagata[2], A. Rasmussen[3], R.P. Grewal[4], K. Watase[2], K. Tsuji[1], M. Khajavi[1], A. McCall[2], C.F. Davis[1], P. Yescas[3], L. Zu[5], S.M. Pulst[5], E. Alonso[3], J.L. Noebels[1,2], D.L. Nelson[2], H.Y. Zoghbi[1,2], T. Ashizawa[1]*. 1) Neurology, Baylor College of Medicine, Houston, TX; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Instituto Nacional de Neurologia y Neurocirugia, Mexico D.F., Mexico; 4) New Jersey Neuroscience Institute, JFK Medical Center, Edison, NJ; 5) Neurology, Cedars-Sinai Medical Center, Los Angeles, CA.

SCA10 is an autosomal dominant disorder characterized by cerebellar ataxia and seizures with anticipation. The SCA10 gene maps to a 3.8 cM interval on chromosome 22q13-qter. Because several other SCA subtypes are associated with trinucleotide repeat expansions, we searched for expanded microsatellites sequences in this region. No triplet repeat expansions were found, however. Using multiple sets of primers flanking a pentanucleotide (ATTCT) repeat in intron 9 of the E46 gene, we found that a polymorphic allele was amplified only from the normal chromosome of affected individuals without amplification of any sequences from the disease chromosome. Southern blot of restriction fragments containing this repeat detected variably expanded alleles, up to 19 kb larger than the normal allele, in all affected individuals in six Mexican families. There was a weak inverse correlation between the expansion size and the age of onset ($r^2 = 0.34$, $p = 0.018$). The expansion size of peripheral blood cells (PBC) differed from that of transformed lymphoblastoid cells of the same patients, and the expanded allele in PBC changed its size with age, suggesting somatic instability of the expanded allele. Thus, the weak inverse correlation between the expansion size and the age of onset may be, in part, attributable to somatic variability of the expanded ATTCT repeat. Analysis of 600 chromosomes from unaffected individuals of various ethnic origins, including Mexicans, showed a size range of 10 to 22 repeats with no evidence of expansions. Our data indicate that the novel intronic ATTCT repeat in SCA10 patients is unstable and represents the largest microsatellite expansion found to date in the human genome.

Program Nr: 233 from the 2000 ASHG Annual Meeting

Human frataxin maintains mitochondrial iron homeostasis in *Saccharomyces cerevisiae*. *P. Cavadini[1], C. Gellera[2], P. Patel[3], G. Isaya[1]*. 1) Departments of Pediatric and Adolescent Medicine and Biochemistry & Molecular Biology , Mayo Clinic and Foundation, Rochester, MN; 2) Division of Biochemistry and Genetics, Istituto Nazionale Neurologico "C. Besta", Milan, Italy; 3) Departments of Neurology and Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Frataxin is a nuclear-encoded mitochondrial protein widely conserved among eukaryotes. Human frataxin, Hfxn, is severely reduced in Friedreich ataxia (FRDA), a frequent autosomal recessive neurocardiodegenerative disease. The vast majority of patients are homozygous for GAA repeat expansions in the first intron of the FRDA locus and show a reduction in the levels of Hfxn ranging between 6% and 30% of control levels. Approximately 4% of FRDA patients carry one GAA expansion and a truncating or missense mutation in the other allele. The mechanism by which Hfxn defects result in the pathology of FRDA has not yet been elucidated. The yeast frataxin homologue (Yfh1p) is involved in mitochondrial iron homeostasis and protection from free radical toxicity. Evidence of iron accumulation and oxidative damage in FRDA heart suggests that Hfxn may have a similar role. We show that a wild-type FRDA cDNA can complement Yfh1p-deficient yeast (*yfh1*D) by preventing the mitochondrial iron accumulation and oxidative damage associated with loss of Yfh1p. We analyze the functional effects of two FRDA point mutations, G130V and W173G, associated with a mild and a severe clinical presentation, respectively. The G130V mutation affects protein stability and results in low levels of mature (m) Hfxn, which are nevertheless sufficient to rescue *yfh1*D yeast. The W173G mutation affects protein processing and stability and results in severe mHfxn deficiency. Expression of the FRDA(W173G) cDNA in *yfh1*D yeast leads to increased levels of mitochondrial iron which are not as elevated as in Yfh1p-deficient cells but are above the threshold for oxidative damage of mitochondrial DNA and iron-sulfur centers, causing a typical *yfh1*D phenotype. These results demonstrate that Hfxn functions like Yfh1p, providing experimental support to the hypothesis that FRDA is a disorder of mitochondrial iron homeostasis.

Program Nr: 234 from the 2000 ASHG Annual Meeting

Bardet-Biedl syndrome is caused by mutations in the MKKS gene, a putative chaperonin*MKKS*. *A. Slavotinek[1], E. Stone[2], J. Heckinlively[3], J. Green[4], E. Heon[5], M. Musarella[5], P. Parfrey[4], V. Sheffield[2], L. Biesecker[1]*. 1) Bldg 49, Rm 4B75, NHGRI/NIH, Bethesda, MD; 2) Dept of Ophth, HHMI and Dept of Peds, University of Iowa, Iowa City; 3) Dept of Ophth, Harbor-UCLA Medical Center, Torrance, CA; 4) Faculty of Medicine, Memorial Univ, St. Johns, Newfoundland; 5) Dept of Ophth, Univ of Toronto (EH); Long Island College Hospital, Brooklyn, NY (MM).

Bardet-Biedl syndrome (BBS) includes pigmentary retinopathy (RP), postaxial polydactyly (PAP), obesity, learning disability and hypogenitalism. BBS has been linked to 5 loci, but no causative gene has been identified. Approximately 8% of BBS patients are unlinked. There is clinical overlap of BBS and McKusick-Kaufman syndrome (MKS), which comprises hydrometrocolpos, PAP and cardiac malformations. We have found mutations in the MKKS gene for MKS on chromosome 20p12 in an Amish family and in a sporadic patient. The overlap of BBS and MKS led us to analyze the MKKS gene in 27 BBS patients, linked BBS families were excluded. Two of the 27 patients with BBS had mutations in MKKS, establishing this gene as the first BBS gene to be cloned and identifying a sixth locus for BBS (BBS6). The first patient had RP, PAP, obesity and retardation. She was a compound heterozygote for 1042G>A transition, G52D and 1679T>A transversion, 264 stop in exon 3 of the MKKS gene. A second BBS patient and her affected brother born to second cousins were homozygous for 1316delC and 1324-1326delGTA in exon 3 of the gene. The proband and her brother had atypical RP, PAP, retardation, and obesity. We conclude that MKS is a phenotypic variant of BBS. The frequency of MKKS mutations in BBS patients conforms to predictions from linkage studies (2/27 = 7.4%; 95% CI 1-24%). The MKKS mutations provide data relevant to the pathology of BBS and may assist in the identification of other BBS genes. We hypothesize that the age dependent features of BBS (RP, growth abnormalities and retardation) are caused by the inability of the MKKS putative chaperonin to maintain protein integrity in the retina, brain, pancreas, and other organs. These results suggest genes encoding chaperonins and their substrates are candidates for other BBS loci, RP, diabetes, obesity and retardation.*MKKS*.

Program Nr: 235 from the 2000 ASHG Annual Meeting

Proposed diagnostic criteria for Stickler syndrome. *P.S. Rose[1,3], H.P. Levy[1], J.J. Johnston[1], J. Davis[1], A.J. Griffith[2], R.M. Liberfarb[1], C.A. Francomano[1]*. 1) NHGRI, National Institutes of Health, Bethesda, MD; 2) NIDCD, National Institutes of Health, Bethesda, MD; 3) Johns Hopkins University School of Medicine, Baltimore, MD.

Stickler syndrome (hereditary arthro-ophthalmopathy) is an autosomal dominant connective tissue disorder caused by mutations in the precursor genes for types II and XI collagen. Prevalence is approximately 1/10,000, and the syndrome is characterized by skeletal, ocular, oral-facial, and auditory manifestations. However, molecular analysis is not generally available, and there are currently no standard established diagnostic criteria for Stickler syndrome.

A diagnostic nosology was created based on the clinical features of 15 individuals diagnosed with Stickler syndrome with known COL2A1 mutations. These criteria were then applied to a cohort of 54 previously evaluated individuals, 36 of whom were considered affected and 18 of whom were thought to be unaffected. Three of the latter 18 were negative for a known COL2A1 mutation segregating in their families.

A 9 point diagnostic scale was established with 1 point for molecular data or positive family history and a maximum of 2 points attainable for characteristic oral-facial, ocular, auditory and skeletal manifestations. A score of 5 or greater was considered diagnostic for Stickler syndrome. These criteria demonstrate 100% sensitivity when applied to the 15 patients with known mutations, 97% sensitivity when applied to the cohort of 36 patients previously diagnosed with Stickler syndrome and 100% specificity when applied to the cohort of 18 patients previously considered unaffected based on clinical and/or molecular analysis. There was no difference in the clinical severity of the 15 patients in the molecularly confirmed cohort and the 36 in the clinically diagnosed group (mean diagnostic scores ignoring molecular/family history data were 6.33 and 6.14 respectively, $p>0.5$).

These diagnostic criteria appear to be sensitive and specific for the diagnosis of Stickler syndrome.

Program Nr: 236 from the 2000 ASHG Annual Meeting

Evaluation of neurofibromatosis 2 (NF2) clinical diagnostic criteria in NF2 patients without bilateral vestibular schwannomas. *M.E. Baser[1], J.M. Friedman[2], A. Wallace[3], R.T. Ramsden[4], D.G.R. Evans[3].* 1) Los Angeles, U.S.A; 2) Department of Medical Genetics, University of British Columbia, Vancouver, Canada; 3) Department of Medical Genetics, St. Mary's Hospital, Manchester, U.K; 4) Department of Otolaryngology, Manchester Royal Infirmary, Manchester, U.K.

Four sets of clinical diagnostic criteria have been proposed for NF2 - the 1987 and 1991 NIH Consensus Conferences criteria, the 1992 Manchester criteria, and the 1997 JAMA criteria - which differ for patients without bilateral vestibular schwannomas (VSs). We compared the proportion of people who met each set of criteria in 85 patients in the United Kingdom NF2 series who presented without bilateral VSs. The patients were divided into (1) a "definite NF2" group: 62 patients who had an identified constitutional *NF2* mutation, or who eventually developed bilateral VSs, or who had a family history of classically-defined NF2, and (2) a "possible NF2" group: 23 sporadic patients with unidentified mutations who had other disease features of NF2. The Manchester criteria had the highest proportion of true positives as defined by the "definite NF2" group (84%). Ninety-six percent of the "possible NF2" group met the Manchester criteria, but additional clinical follow-up and mutation identification is needed to further document disease status in this group. Minor modifications to the Manchester criteria can increase identification of true positives (e.g., requiring only one tumor or cataract, as in the 1991 NIH criteria, increases the true positives to 89%).

Program Nr: 237 from the 2000 ASHG Annual Meeting

Natural history of branchio-oto-renal (BOR) syndrome. *L. Hudgins[1], M.C. Jones[2], R.S. Olney[1], G.M. Enns[1], S.L. Schelley[1]*. 1) Pediatrics/Med. Gen., Stanford Univ, Stanford, CA; 2) Dysmorphology/Genetics, Children's Hospital, San Diego, CA.

The association of renal malformations and external ear anomalies/hearing loss in BOR has been well-described, however, the morbidity and outcome of these abnormalities have been less well-defined. Utilizing the published diagnostic criteria for BOR, we identified 6 families with this condition (4 4-generation, 1 3-generation, and 1 2-generation). Medical records were reviewed including all available audiograms and imaging studies. A total of 48 individuals including 3 phenotypically normal obligate gene carriers were included, 29 females and 19 males. Hearing loss was the most common finding seen in 32 individuals (67%), followed by ear pits in 31 (65%), branchial cysts in 20 (42%), external ear anomalies in 17 (35%), structural renal anomalies in 9 (19%), and lacrimal duct stenosis in 7 (15%). All types of hearing loss were identified including sensorineural, conductive, and mixed, most commonly bilateral (91%) and occasionally progressive. In the 17 individuals identified with external ear anomalies, 12 had one or more cupped ears, 3 had overfolded helices ("lop" ears), and 3 had either unilateral or bilateral microtia. Small kidneys and mild pelviectasis were each seen in 3 individuals, with horseshoe kidney in 2 and unilateral agenesis in 1. Abnormal renal function was noted in one newborn, which resolved, and one 2 y.o. who was lost to follow-up. Both of these individuals had small kidneys. UTI's were described in 3 individuals, only one of whom had renal imaging which was normal. Nephrolithiasis was noted in one older individual with structurally normal kidneys. More importantly, of the 48 individuals including several older persons in four 4-generation pedigrees, none had any significant morbidity associated with the urogenital system. We conclude that although there is an association between external ear anomalies/ preauricular pits and structural renal abnormalities in BOR syndrome, the morbidity for renal anomalies is low. Renal imaging may be of academic interest, but even if structural anomalies are present, prognosis for normal renal function is good.

Program Nr: 238 from the 2000 ASHG Annual Meeting

Natural history of Jarcho-Levin syndrome. *A.S. Cornier[1], N. Ramrez-Lluch[2], S. Arroyo[2], A. Marquez[3], J. Acevedo[4], M. Warman[5]*. 1) Dept Biochemistry, Division of Genetics, Ponce Sch Medicine,Ponce, P.R Ponce, PR; 2) Department of Ped. Orthopedics and Department of Radiology, Mayaguez Medical Center, Mayaguez, P.R; 3) X-Ray Diagnostic Center, Mayaguez, P.R; 4) Department of Nursing, University of Puerto Rico at Arecibo, Arecibo, P.R; 5) Department of Genetics, Case Western Reserve University, Cleveland, OH.

Jarcho-Levin syndrome (JLS, MIM#277300) is an autosomal recessive disorder with high prevalence in Puerto Rican population that has been described as a lethal condition. Since Jarcho and Levin described it in 1938, has been referred to as spondylocostal dysplasia, costovertebral dysplasia, spondylothoracic dysplasia and dysostosis. We has prospectively characterized 16 patients with spondylothoracic dysostosis, which is what initially Jarcho and Levin described. We have performed detail physical examination, pedigree analysis, chest X-rays, chest CT Scans and pulmonary function tests(PFT's). Diagnosis of the disorder was made by chest X-ray and 3-D reconstructive CT scans that showed fusion of the ribs at the costo-vertebral junction with a fan like configuration of the thorax. Numerous vertebral anomalies were found. Six out of these 16 patients has been born during our study and three of them survived to 12, 16 and 32 months respectively. The cause of death in diseased patients was respiratory insufficiency secondary to restrictive lung problems and pneumonia. Age of the remaining patients ranges from 7 to 40 years. PFT's performed on individuals older than 6 years showed a severe restrictive pattern that seems not to exacerbate as patients progresses in age. Mortality of our kindred was 50% of identified newborns with JLS. This is an important finding since the vast majority of the JLS patients cited in the medical literature died early in the newborn or early childhood period. Their intellectual capacity and development is normal in all cases. This is the largest kindred of patients with JLS described in the medical literature and so forth has allowed us to determine a detail phenotype and natural progression of the disease. A genome-wide linkage analysis is undergoing to determine the locus (i) of the JLS in Puerto Rican population.

Program Nr: 239 from the 2000 ASHG Annual Meeting

Acromicric dysplasia: long-term outcome and evidence of autosomal dominant inheritance. *L. Faivre[1], M. Le Merrer[1], C. Baumann[2], P. Maroteaux[1], A. Munnich[1], V. Cormier-Daire[1].* 1) Dept Genetics, Hopital Necker Enfants Malades, Paris, France; 2) Dept Genetics, Hopital Robert Debre, Paris, France.

In 1986, P. Maroteaux described a novel bone dysplasia in six unrelated children, presenting with short stature, short hands and feet, normal intelligence, characteristic X-ray abnormalities of the hands, and he coined this condition acromicric dysplasia. In the last 14 years, we have ascertained 17 additional cases of acromicric dysplasia in 14 families from 5 different countries (13 girls, 7 boys), and followed three of the previously reported cases. The diagnosis of acromicric dysplasia was based on the observation of 1) short stature 2) short hands and feet 3) short and stubby metacarpals and phalanges with presence of an external notch on the 2nd metacarpal and an internal notch on the 5th metacarpal and cone-shaped epiphyses of the phalanges, 4) absence of spondylo-epi-metaphyseal dysplasia, and 5) absence of storage disease. Patients ranged from age 4 to 53 years. The mean height at birth was 48 cm and the mean adult height was 132 cm. Reduced postnatal growth speed was a consistent feature in our series. In addition, 15/20 cases had a dolly-like appearance, 5/20 cases had a heart malformation and 2/20 had ophthalmologic abnormalities. In the older patients, arthrosis, spine abnormalities, carpal tunnel syndrome and tracheal abnormalities occurred. The disease appeared sporadic in 14 cases but the observation of vertical transmission in three families was consistent with an autosomal dominant inheritance.

Several patients presenting with features of acromicric dysplasia, stiff joints, thickened skin and hepatomegaly have been reported in the literature, raising the question of whether geleophysic dysplasia and acromicric dysplasia could be the same entity despite different modes of inheritance. Interestingly, the oldest patient of our series has recently developed cardiac valve infiltration, hepatomegaly and tracheal infiltration, which are features of geleophysic dysplasia. This observation suggests that these two syndromes are either allelic forms of the same disorder or result from different alterations of the same metabolic pathway.

Program Nr: 240 from the 2000 ASHG Annual Meeting

Diagnostic integrity of the MASS phenotype and natural history of the cardiovascular features. *R.E. Pyeritz[1,2], D.L. Thull[2], M.K. Bourdius[2], S.L. Sell[2].* 1) MCP Hahnemann University, Pittsburgh, PA; 2) Allegheny General Hospital, Pittsburgh, PA.

Marfan syndrome (MFS) lies at the severe end of a phenotypic continuum of connective tissue disorders. In 1989, a group of patients along this continuum who were both less severe than MFS and, based on a cross-sectional perspective, less likely to progress were defined as MASS phenotype. Cardiovascular features of MASS include mitral valve prolapse (MVP) and minimal or no aortic root dilatation. We studied 82 patients with MASS ascertained between 1994 and 1999, to determine whether the revised diagnostic criteria for MFS have affected diagnosis of MASS, and if cardiovascular features are progressive. From medical records we recorded baseline phenotypic data and ascertained whether patients met criteria for MFS. Aortic root dimensions (ARD), MVP and severity of mitral regurgitation (MR) were obtained from the original 2-D echo/Doppler tapes. Of the 82 patients originally diagnosed as MASS, none met the current criteria for MFS. Personal follow-up medical evaluation was available on 29 (35%), and none met criteria for MFS or another connective tissue disorder. On initial echo, 18/36 children and 42/52 adults had MVP; 1/36 children and 9/52 adults had MR of moderate severity or worse. On follow-up, 3/5 children developed MVP and 1/12 developed moderate MR; 2/3 adults developed MVP and 5/14 developed moderate MR. Two adults required MV repair. The mean initial ARDs for all ages were greater than average for body size but never 3 SD > the mean. Over mean follow-up of 3.5 y, 4 children had progressive ARD, but at a rate consistent with growth. Four adults had minimal increase in ARD, but none >10%, during mean follow-up of 4.6 y. We conclude that: the diagnosis of MASS has remained valid and stable; progression of MV dysfunction is common at any age; and for patients of any age diagnosed with MASS, dilatation of the aortic root is unlikely to progress over a period of 3-5 years. These data should assist in advising patients as to life style, use of b-blockade, and the interval for periodic clinical and echo evaluations.

Program Nr: 241 from the 2000 ASHG Annual Meeting

Kabuki syndrome patients with coloboma initially diagnosed as CHARGE association: Report of two patients and review of ophthalmologic findings. *J.E. Ming, K.L. Russell, E.H. Zackai.* Division of Human Genetics, The Children's Hospital of Philadelphia and The Univ. of Pennsylvania School of Medicine, Philadelphia, PA.

Kabuki syndrome features developmental delay, congenital heart disease, and characteristic facial features. We report two children with Kabuki syndrome who had retinal colobomas, resulting in initial diagnostic uncertainty before the typical features of Kabuki syndrome emerged. The first patient was seen in infancy at another center and was noted to have bilateral retinal colobomas, growth failure, ventricular septal defect, anomalous ears, hearing loss, and small kidneys. A diagnosis of CHARGE association was suggested. At the age of 14 months, we diagnosed him with Kabuki syndrome since at that time typical features including prominent eyelashes, lateral lower eyelid eversion, and fingertip pads were apparent. The second patient had left iris and retinal coloboma, atrial septal defect, abnormal ears, and cleft palate. CHARGE association was considered in the neonatal period. On follow-up at 2 years, he was noted to have the typical facial features of Kabuki syndrome. Including these two patients, we have diagnosed 11 patients with Kabuki syndrome, of whom 6 had a documented ocular anomaly, including strabismus, ptosis, nystagmus, and lacrimal duct obstruction. Review of recent reports have revealed an incidence of ocular findings in 33%-61% of patients. In these reports, we noted four patients with a coloboma, and findings in addition to those present in our patients included corneal abnormalities, Duane syndrome, Peters anomaly, and optic nerve hypoplasia. Thus, ophthalmologic abnormalities are frequently associated with Kabuki syndrome, and an ophthalmologic evaluation should be performed for each patient. Phenotypic overlap can lead to the erroneous diagnosis of CHARGE association, especially since many of the typical features of Kabuki syndrome may not be apparent in early infancy. Thus, Kabuki syndrome should be considered in patients with coloboma if other features consistent with this condition are present, and follow-up evaluations are indicated for establishing the proper diagnosis.

Program Nr: 242 from the 2000 ASHG Annual Meeting

Severe early infantile onset of developmental retardation, cerebellar hypoplasia, and seizures in an infant with a maternally inherited extreme CAG-repeat expansion in the Spinocerebellar Ataxia Type 2 (SCA2) gene. *A.S. Aylsworth[1], R. Mao[2], W.G. Wilson[3], K. Kaiser-Rogers[1], M.B. Tennison[1], L.M. Silverman[1], K. Snow[2], N.T. Potter[4].* 1) Univ North Carolina, Chapel Hill; 2) Mayo Clinic, Rochester MN; 3) Univ Virginia, Charlottesville; 4) Univ Tennessee, Knoxville.

SCA2 usually presents between age 20 and 40 years with progressive symptoms including ataxia of the trunk and limbs, dysarthria, slow ocular saccades, ophthalmoplegia, and, in some, peripheral neuropathy and early dementia. We have studied an African-American baby with very early and severe infantile onset of symptoms. CLINICAL FEATURES include: encephalopathy with severe developmental retardation, hypertonic extremities, cerebellar atrophy, visual impairment, microcephaly, and infantile spasms unresponsive to ACTH; chronic megaloblastic anemia; short stature; hyperglycemia; and persistent hyponatremia and hyperkalemia. FAMILY HISTORY includes three ancestral generations with onset of cerebellar ataxia and dysarthria in the 3rd and 4th decades. Relatives affected include his mother, maternal uncle, grandfather, great-uncle, and great grandmother. MOLECULAR ANALYSIS showed an extremely large expansion of ~350 CAG-repeats in SCA2. His mother has 45 repeats. To improve detection of expansions of >150 CAG repeats, we used an assay based on agarose gel separation of PCR products, blotting, and hybridization with a $(CAG)_6$ probe. DISCUSSION: Paternally inherited extreme expansions in SCA2 have been observed in three other patients with early infantile onset of neurologic symptoms [Am J Med Genet 79:383, 1998; J Molec Diagnostics 1:43(abstract G19), 1999; Mao & Snow, ms. in prep.]. This is the first case observed with a maternally inherited extreme expansion. The causes of this patient's chronic anemia and other non-neurologic signs are as yet unexplained, but may be significant in broadening the phenotype associated with extreme CAG-repeat expansions in SCA2. This case also illustrates how an extreme expansion might be missed by routine testing protocols. When "homozygous normal" results are obtained for a proband with juvenile or infantile onset SCA, we strongly recommend molecular testing for the parents.

Program Nr: 243 from the 2000 ASHG Annual Meeting

Fragile X mosaics in a family with multiple mildly affected individuals. *M.M. Li[1], L. Nelson[1], M. Bamshad[2], K. Ward[1]*. 1) Perinatal Genetics/DNA Diagnostic Laboratory, University of Utah Medical Center, Salt Lake City, UT; 2) Department of Pediatrics, University of Utah Medical Center, Salt Lake City, Utah.

Fragile X syndrome is the most common form of inherited mental retardation. It is caused by a CGG triplet expansion in the 5' untranslated region of exon one of the FMR1gene. Mosaic triplet expansion is observed in about 20-40% of male patients. Here we report a fragile X family with both expansion size mosaicism and methylation mosaicism. Two brothers (ML and DL) and their maternal cousin (PO) were referred for fragile X testing because of speech delay. Their medical histories were notable for frequent otitis media and upper respiratory infections. Although they require tutorial assistance, they are able to attend school with other students of their age. A family history of learning difficulties and mental retardation in some of the maternal relatives was noted as well. Physical exams revealed that all three patients had a long face and mild developmental delay. Both ML and DL showed hyperextensibility of their distal joints. PO had been previously diagnosed with autism. Molecular studies using both PCR and southern blot techniques were performed on these patients and their mothers. Both brothers were found to be mosaics for the CGG triplet expansion (87 and 300-500 repeats for ML; 53 and 200-250 for DL). PO had a full mutation of about 200 to 300 repeats with 70% methylation (methylation mosaics). The full mutations in ML and DL were fully methylated. The mothers were premutation carriers (90 and 75 repeats). These results suggest that the partial methylation of the full CGG expansion in the FMR1 gene in PO and the premutations in ML and DL may have spared them from mental retardation, the most serious effect of the condition. Alternatively, tissue mosaicism (i. e. in the brains of ML and DL) could be responsible for their functioning at higher levels. Studies of methylation pattern in skin or buccal cell samples may answer this question. Our data emphasize that PCR based tests for the diagnosis of fragile X should always be accompanied by southern blot analyses to eliminate any possibilities of false negative results due to the high level of mosaicism in male patients.

Program Nr: 244 from the 2000 ASHG Annual Meeting

Autosomal recessive severe intraosseous hemangioma in the skull. A new syndrome? *A.N. Akarsu[1], I. Vargel[2], Y. Erk[2]*. 1) Departments of Pediatrics and; 2) Plastic and Reconstructive Surgery, Hacettepe University Ankara, Turkey.

Intraosseous hemangioma is a rare, slowly growing, benign tumor that accounts for 0.2% of all bone tumors. It is commonly found in the vertebrae and skull, especially in the mandibula and maxilla. Although it is considered as benign tumor this condition is extremely serious especially in jaw bones and in several instances the simple procedure of tooth extraction may have a fatal ending due to profound bleeding. It is exclusively described as sporadic cases and no hereditary component has been reported so far. Terminology and classification of hemangiomas are confusing. Number of classification have been proposed however, none of them could cover all types of hemangiomas and vascular malformations. According to widely used classification developed by Mulliken and Glowacki, vascular anomalies can be divided into two major categories (hemangiomas and vascular malformations) due to their clinical behaviour and endotelial cell characteristics. Hemangiomas rarely affect bone whereas malformations affect bone in 35% of cases. It has been suggested that lesions involving bone are almost certain to be malformations rather than hemangiomas and true intraosseous hemangiomas are extremely rare, if they occur at all. Here, we report four case from two unrelated consanquineous families manifesting severe, progressive intraosseous hemangioma affecting almost all bones in the skull associated with midline defects such as diastasis recti, umblical hernia and raphe. Abnormality first occures in the mandibular bone and all cranial bones are gradually involved. Radiographic evaluation revealed soap-bubble effect; pathologic diagnosis indicated centrale intraosseous hemangioma. Angiography did not reveal any gross arteriovenous malformation. Despite dramatic feature of the cases no soft tissue involvement, pulsation or trill supporting vascular malformations have been detected. Inbred nature of both families suggested autosomal recessive mode of inheritance and this is the first report of hereditary form of intraosseous hemangioma in the skull. Homozygosity mapping excluded number of candidate genes on chromosomes 4, 7, 10 and 12.

Program Nr: 245 from the 2000 ASHG Annual Meeting

Somatic deletions in hereditary breast cancers implicate 13q21-q22 as a putative novel breast cancer susceptibility locus*. *S.H Juo.* for the Nordic-NHGRI Hereditary Breast Cancer Consortium*.

A significant proportion of familial breast cancers cannot be explained by mutations in the BRCA1 or BRCA2 genes. We applied a new strategy to identify predisposition loci for breast cancer using mathematical models to identify early somatic genetic deletions in tumor tissues followed by targeted linkage analysis. Comparative genomic hybridization (CGH) was used to study 61 breast tumors from 37 breast cancer families with no identified BRCA1 or BRCA2 mutations. Branching and phylogenetic tree-models predicted that loss of 13q was one of the earliest genetic events in hereditary cancers. In a Swedish family with five breast cancer cases, all analyzed tumors showed distinct 13q deletions, with the minimal region of loss at 13q21-q22. Genotyping revealed segregation of a shared 13q21-q22 germ-line haplotype in the family. Targeted linkage analysis was carried out in a set of 77 Finnish, Icelandic and Swedish breast cancer families with no detected BRCA1 and BRCA2 mutations. A maximum parametric two-point LOD score of 2.76 (at $q = 0.10$) and a multipoint lod score of 3.46 under heterogeneity were obtained. The results were further evaluated by simulation to assess the probability of obtaining significant evidence in favor of linkage by chance as well as to take into account the possible influence of the BRCA2 locus, located ~25 cM proximal on the same chromosome from the new locus. The simulation substantiated the evidence of linkage at 13q21-q22 ($p < 0.0017$). The results warrant studies of this putative breast cancer predisposition locus in other populations. *Members of the consortium: CGB and IDRB at NHGRI, NCBI, DKFZ, Tampere University and Helsinki University Hospitals, Finland, University Hospital of Iceland, Reykjavik, Iceland, Lund University Hospital, Sweden.

Program Nr: 246 from the 2000 ASHG Annual Meeting

A partial BRCA1 sequence homology on 4q28. *I. Stec[1], M. van Vliet[2], R. van Eijk[1], J.T. den Dunnen[1], K.H.G. Kroeze[1], G.-J.B. van Ommen[1], C.J. Cornelisse[3], P. Devilee[1]*. 1) MGC-Dept of Human and Clinical Genetics, Leiden University Medical Center (LUMC), The Netherlands; 2) Dept of Clinical Genetics, Erasmus University Rotterdam, The Netherlands; 3) Dept of Pathology, LUMC, The Netherlands.

We have identified a sequence on chromosome 4q28 which is for 99% homologous to the 3' end of the human BRCA1 gene. The homology was detected as a cross-hybridising 3.7kb *Bgl*II fragment in Southern analysis, using a cDNA-probe of BRCA1 containing exons 14-24 (p1424). Southern analysis of a human/rodent chromosome hybrid mapping panel suggested this fragment mapped to chromosome 4. Two partially overlapping cosmids were isolated after screening a chromosome 4 cosmid library using p1424. These cosmids mapped to 4q28 in FISH analyses. After subcloning and sequencing the cosmids, a contig has been assembled spanning both the BRCA1 homology and the 3.7kb *Bgl*II fragment. An ORF was identified encoding a sequence of 82 amino acids, the last 23 of which are (except one) identical to the last 23 of BRCA1. Strikingly, the upstream *Bgl*II-site was shown to be variant in 5 small Dutch breast cancer families without BRCA1/2 mutations, in 5 out of 376 unselected sporadic breast cancer patients (1.3%) and in 3 out of 282 healthy blood donors (1.1%). We detected the variant in 5 out of 136 additional breast cancer patients with a positive family history (3.6%) (P-value of 0.07 comparing the latter two groups). This suggested that this *Bgl*II-site might be located within or upstream to a new breast cancer susceptibility gene. Subsequently, the variant was also detected in 6 out of 266 female controls (2.2%) who had reached the age of 85 in good health. Nineteen bp next to the *Bgl*II variant, a G to A change was detected which was present, on the same allele, in all *Bgl*II variant carriers except in one lady aged >85. Until now, we have not been able to detect any matches in dbEST besides some retrovirus related sequences of which it is unclear whether they derive from this particular region, nor have we isolated expressed sequences by RT-PCR or RACE. The status of a putative gene and its sequence variants remain unclear and may require further collaboration between groups with similar findings.

Program Nr: 247 from the 2000 ASHG Annual Meeting

Screening for Genomic Rearrangements in Breast and Ovarian Cancer Families Identifies BRCA1 Mutations Previously Missed by CSGE or Sequencing. *H.X Zhang[1], M.A Unger[1], K.L Nathanson[1], D. Antin-Ozerkis[1], H. Shih[1], A.M Martin[1], K. Calzone[1], G.M Lenoir[2], S. Mazoyer[2], B.L Weber[1].* 1) Department of Medicine, University of Pennsylvania, Philadelphia, PA; 2) International Agency for Research on Cancer, 69372 Lyon cedex 08, France.

The frequency of genomic rearrangements in BRCA1 was assessed in 42 American breast and ovarian cancer families known to be negative for BRCA1 and BRCA2 coding-region mutations. An affected individual from each family was tested by PCR for the exon 13 duplication (Puget et al.(1999), Amer J Hum Genet 64:300-302) and also for novel genomic rearrangements by Southern blot analysis. The exon 13 duplication was detected in one family and an additional 4 families had other genomic rearrangements. In total, 11.9% (5/42) of breast-ovarian families without BRCA1 and BRCA2 coding-region mutations had mutations in BRCA1 that were missed by CSGE or sequencing. Furthermore, 4 out of the 5 families (80.0%) that had BRCA1 genomic rearrangements had at least one individual with both breast and ovarian cancer. Therefore, in the 13 families in this study that contain an individual with both breast and ovarian cancer the frequency of a detectable genomic rearrangement in BRCA1 was 30.8% (4/13). By way of comparison, these data suggest that genomic rearrangements in BRCA1 occur with the same frequency as BRCA2 coding-region mutations in non-Ashkenazi Jewish breast-ovarian cancer kindreds. This study gives evidence that a significant portion of BRCA1 mutations are missed by failure to screen for genomic rearrangements, particularly in families that contain an individual with both breast and ovarian cancer.

Program Nr: 248 from the 2000 ASHG Annual Meeting

Immunoassay predicts BRCA1 and BRCA2 mutations in buccal cells. *T.J. Byrne[1], M.T. Reece[2], L.A. Adams[2], D.E. Hoffman[2], M.A. Lane[2], G.M. Cohn[2].* 1) University of Massachusetts, Amherst, MA; 2) Department of Obstetrics and Gynecology, Baystate Medical Ctr, Springfield, MA.

Approximately 5-10% of breast and ovarian cancers are heritable and 4-5% of unaffected, reproductive age women are at significant for heritable breast and/or ovarian cancer. BRCA1 and BRCA2 (BRCA) mutation analysis is used in the clinical triage of at risk individuals, however, the cost of such testing limits its routine use. We have previously reported the development of an inexpensive, antibody assay which detects loss of heterozygosity in ovarian tumors and protein truncation mutations in non-tumor surgical specimens and buccal cells. This immunohistochemical assay employs antibodies directed at either end of the BRCA proteins and predicts the presence of BRCA gene alteration in the presence of diminished anti-carrboxy immunoreactivity relative to anti-amino immunoreactivity. The aim of this study was to further determine the sensititivity, specificity, and predictive values of this assay. Buccal cells and peripheral blood were collected from 5 at risk individuals. Immunohistochemical analysis using anti-carboxy and anti-amino BRCA1 and BRCA2 antibodies was performed. The presence of diminished immunoreactivity for either protein was scored as predictive for mutation. This analysis was compared to the results of BRCA DNA analysis. To date we have analyzed 16 high risk individuals. All 11 BRCA germline mutations have been correctly predicted using this immunoassay. Of 5 individuals with no detectable BRCA mutations, 4 were correctly predicted as being negative by immunoassay. Among high risk individuals, the sensitivity, specificity, positive, and negative predictive values were 100%, 80%, 92%, and 100%, respectively ($p<0.003$). These results suggest that the buccal, BRCA immunoassay shows great promise as an inexpensive screen for BRCA mutations.

Program Nr: 249 from the 2000 ASHG Annual Meeting

Genetic modifiers of BRCA1 penetrance. *K. Nathanson[1], Y. Yao[3], C. Szabo[4], R. Omaruddin[1], P. Devilee[5], T. Rebbeck[2], B. Weber[1]*. 1) Dept Medicine, Univ Penn Sch of Med, Phila, PA; 2) Dept Biostat & Epi, Univ Penn Sch of Med, Phila, PA; 3) CIDR, Johns Hopkins Univ, Baltimore, MD; 4) IARC, Lyon, France; 5) LUMC, Leiden, Netherlands.

Women with germline mutations in BRCA1 have a greatly elevated risk of breast and ovarian cancer. However, considerable variation in the degree of breast cancer risk has been observed, suggesting that genetic modifiers of BRCA1 penetrance may exist. We evaluated 1) whether there is evidence for modifier loci, and 2) whether modifiers can be identified using non-parametric linkage analyses (NPL). In order to determine whether modifiers of breast cancer penetrance exist, 894 relative pairs of BRCA1 mutation carriers were studied for correlation of breast cancer incidence. Preliminary analyses suggested that breast cancer risk is correlated among biological relatives overall (r=0.122, p=0.002), but that BRCA1 mutation status did not completely explain breast cancer case status, even among relatives who carried the 185delAG mutation (r=0.675, p=0.001). Thus, residual family correlation of breast cancer risk may exist that is not explained by the BRCA1 mutation alone. In order to identify modifier genes, we used NPL methods to examine if haplotypes are more frequently shared in BRCA1 mutation carriers affected with breast cancer than would be expected by chance alone. For this analysis we used 19 families: 17 families with deleterious mutations in BRCA1 and 2 families with a >90% posterior probability of carrying a mutation in BRCA1. We hypothesized that the modifier genes might be located in chromosomal regions lost in the tumors of BRCA1 mutation carriers, documented by CGH (4p, 4q, 5q). We did not observe any regions on 4p or 4q that were associated with breast cancer risk in BRCA1 mutation carriers. However, we observed a maximum multi-point NPL score on chromosome 5q of 2.23 (p=0.02), which increased to 3.10 (p=0.005) in families with an average age of breast cancer diagnosis (AAD) <45 years, and to 3.16 (p=0.006) in families with AAD <45 and BRCA1 mutation in exons 1-12. These results suggest the presence of a gene(s) on chromosome 5q that modify breast cancer risk in BRCA1 mutation carriers.

Program Nr: 250 from the 2000 ASHG Annual Meeting

Reduction in Breast Cancer Risk Following Bilateral Prophylactic Oophorectomy in BRCA1 and BRCA2 Mutation Carriers. *A. Eisen[1], T.R. Rebbeck[1], H.T. Lynch[2], C. Lerman[3], P. Ghadirian[4], M.P. Dubé[5], B.L. Weber[1], S.A. Narod[5]*. 1) University of Pennsylvania School of Medicine, Philadelphia, PA; 2) Creighton University, Omaha, NE; 3) Georgetown University Medical Center, Washington, DC; 4) Epidemiology Research Unit, Centre Hospitalier Universitaire Montréal,Montréal,QC; 5) Women's College Hospital, Toronto,ON.

Women with BRCA1 or BRCA2 mutations have a lifetime risk of developing breast cancer that may reach 80%. BRCA1 mutation carriers who have undergone prophylactic oophorectomy (or oophorectomy for other reasons) have a marked reduction in this risk (Rebbeck et al, JNCI 1999;91:1475-9). In this follow-up study we have estimated the magnitude of the risk reduction associated with oophorectomy at different ages in BRCA1 and BRCA2 mutation carriers. Cases were women with breast cancer and a BRCA1 or BRCA2 mutation. Controls were matched to cases one to one by year of birth (+/- 3 years) and by mutation (BRCA1 or BRCA2). Cases and controls with ovarian cancer were excluded. Overall, oophorectomy was associated with an odds ratio (OR) for breast cancer of 0.42 (95% CI 0.24-0.75). The risk was reduced in both BRCA1 (OR=0.39, 95% CI = 0.20-0.75) and BRCA2 carriers (OR = 0.56, 95% CI = 0.16 to 1.95). The risk reduction was greatest for oophorectomy performed before age 40 (OR=0.24, 95% CI 0.10-0.60), was intermediate for women with oophorectomy between ages 40 and 49 (OR = 0.60;95% CI = 0.26 - 1.41) and was least for oophorectomy performed after age 50 (CI = 0.94, 0.19-4.67). These data show that early oophorectomy should be considered as a possible strategy to reduce breast cancer risk in BRCA1 and BRCA2 mutation carriers.

Program Nr: 251 from the 2000 ASHG Annual Meeting

Ovarian Cancer Risk Reduction after Bilateral Prophylactic Oophorectomy (BPO) in BRCA1 and BRCA2 Mutation Carriers. *B.L. Weber[1], C. Punzalan[1], A. Eisen[1], H.T. Lynch[2], S.A. Narod[2], J.E. Garber[3], C. Isaacs[3], M.B. Daly[4], S.L. Neuhausen[5], T.R. Rebbeck[1]*. 1) U. of Pennsylvania, Philadelphia, PA; 2) Creighton U., Omaha, NE, or Women's College Hospital, Toronto, ON; 3) Dana Farber Cancer Institute, Boston, MA, or Georgetown U., Washington, DC; 4) Fox Chase Cancer Center, Philadelphia, PA; 5) U. of Utah, Salt Lake City, UT.

The availability of genetic testing for inherited mutations in BRCA1 and BRCA2 (BRCA1/2) provides potentially valuable information to women at high risk of breast or ovarian cancer. However, the clinical options for cancer risk reduction to these women are limited, and include bilateral prophylactic oophorectomy (BPO). We have previously reported that BPO is associated with a reduction in breast cancer risk. To evaluate whether BPO is associated with a reduction in ovarian cancer risk, a cohort of women with disease-associated germline BRCA1/2 mutations was assembled from five North American centers. The 248 BPO subjects included women who underwent BPO and had no history of ovarian cancer at the time of surgery. BPO subjects were compared with 245 control women matched on cancer status at the time of the BPO subject's surgery, ascertainment center, locus of mutation, and year of birth. The data consisted of 4403 person years of post-BPO follow-up, with an average post-BPO follow-up of 9.4 years. Of the 248 BPO subjects, 1 woman (0.4%) developed ovarian cancer after BPO, and five women (2%) were diagnosed with ovarian cancer at the time of BPO. Excluding those women diagnosed at the time of surgery, we report a highly significant reduction in ovarian cancer risk after BPO, with an adjusted HR=0.02 (95%CI: 0.002-0.12). In addition, 25 women (10.1%) developed breast cancer after BPO. Overall, the HR associated with breast or ovarian cancer risk reduction was 0.46 (95%CI: 0.29-0.73). Our results indicate that BPO significantly reduces breast and ovarian cancer risk in women who carry a BRCA1/2 mutation, and has important implications for the management of breast and ovarian cancer risk in these women.

Program Nr: 252 from the 2000 ASHG Annual Meeting

The RAD51-135C SNP modifies breast and ovarian cancer risk in BRCA2 carriers, but not in BRCA1 carriers.

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BRCA1 and BRCA2 carriers have a clearly elevated risk for both breast and ovarian cancer, but estimates of lifetime risk vary widely. For breast cancer, risk estimates range from 36% to 85%, depending in part on ascertainment. These differences suggest that BRCA1/BRCA2 penetrance is modified by other genetic and/or environmental factors. The BRCA1 and BRCA2 proteins play a role in DNA repair via homologous recombination, as part of a macromolecular complex which includes RAD51. A single nucleotide polymorphism (SNP) in the 5'UTR of RAD51 (135C/G) has been reported to increase breast cancer risk in BRCA1 and BRCA2 carriers (Wang et al, 1999). In this study we further investigate this effect.

The study group included 257 female Ashkenazi Jewish (AJ) carriers of one of the common BRCA1 (185delAG, 5382insC) or BRCA2 (6174delT) mutations. There were 164 women affected with breast and/or ovarian cancer and 93 unaffected. Among BRCA1 carriers RAD51-135C frequency was similar in healthy and affected women (6.1% (3/49) and 9.9% (12/121) respectively). Using survival analysis, RAD-135C did not influence age of cancer diagnosis in BRCA1 carriers (Hazard ratio=1.16 for disease in RAD51-135C heterozygotes, NS). However, in BRCA2 carriers, RAD51-135C heterozygote frequency in affected women 17.4% (8/46) was higher that in healthy women 4.9% (2/41) (p=0.07). In addition, survival analysis showed that among BRCA2 carriers, age of cancer diagnosis was significantly younger in RAD51-135C heterozygotes: Hazard ratio 4.66 (p=0.0002). Population frequency of RAD51-135C in AJ was 8.2%. These results show RAD51-135C is a clinically significant modifier of BRCA2 penetrance. Its effect on Rad51 expression is being investigated to provide biological support for this epidemiological evidence.

Program Nr: 253 from the 2000 ASHG Annual Meeting

_TP53_ mutations in _BRCA1_ and _BRCA2_-related breast cancer: distinctive spectrum and structural distribution.

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Using computational methods of mutational spectrum analysis and structural modeling, we studied in detail the somatic *TP53* mutations spectrum and their structural distribution in *BRCA1/2*-related breast cancers. All studies providing data on the association between *TP53* mutation status and *BRCA1/2*-related breast cancer were reviewed. The IARC version of the *TP53* somatic mutation database was used for comparison of mutational spectrum. Rendering and visualization of the p53 structure was performed with the InsightII molecular modeling environment program. Appropriate statistical methods were used to compare mutation spectra and spatial distribution of the *TP53* mutations between *BRCA1/2*-related breast cancer and non-hereditary breast cancer. Somatic *TP53* abnormalities were more common in *BRCA1*- and *BRCA2*-associated breast cancers than in sporadic breast cancers (odds ratio: 2.8; 95% CI: 1.6-4.7; $P = 0.0003$). We compared the spectrum of *TP53* mutations for breast cancers in the IARC *TP53* mutation database with the 82 mutations reported in *BRCA1/2*-associated cancers. The spectrum differed significantly both in distribution ($P < 1x10^{-6}$) and in base changes ($P = 0.025$). Mutations at A:T base pairs were more common in *BRCA1/2*-associated tumors, and strand bias and tandem mutations suggesting DNA repair abnormalities were found. Changes were common at *TP53* codons that are not mutation hotspots. Notably, structural modeling showed that most of these nonhotspot amino acids cluster in a region of the p53 protein on the anti-DNA side.

These results suggest that 1) *BRCA1/2* mutations influence the type and distribution of *TP53* mutations seen in breast cancer, and 2) mutations in a region on the opposite side from the p53 DNA binding surface may have important functional consequences in *BRCA1/2*-associated breast cancers, possibly through interaction with an unknown protein.

Program Nr: 254 from the 2000 ASHG Annual Meeting

Genetic Factors Related to Racial Variation in Plasma Levels of Insulin-like Growth Factor-1 (IGF-I). *H. Jernstrom[1,2], W. Chu[1], D. Vesprini[1], Y. Tao[3], N. Majeed[3], C. Deal[4], M. Pollak[3], S.A. Narod[1]*. 1) Centre for Research in Women's Health, Toronto, Canada; 2) Dept of Oncology, Lund University Hospital, Sweden; 3) Lady Davis Research Institute of Jewish General Hospital and McGill Unviersity, Montreal, Canada; 4) Department of Pediatrics and Sainte Justine Hospital, Universite de Montreal, Canada.

Oral contraceptives are associated with a modest increase in breast cancer risk in the general population. Particular subgroups appear to be more susceptible, i.e. higher risks have been reported for black women than for white women. Circulating IGF-I levels vary between ethnic groups and are correlated with premenopausal breast cancer risk. Some women demonstrate high IGF-I levels during periods of pill use and may be at increased risk of premenopausal breast cancer. We measured IGF-I and IGFBP-3 plasma levels in 505 nulliparous women aged 17 to 35. All women filled out a questionnaire including information on ethnic background and pill use. DNA samples were used to genotype the women for known polymorphic variants in the IGF1, AIB1 and CYP3A4 genes. Black women had significantly higher mean IGF-I levels than white women (330 vs 284 ng/ml; p = 0.001, adjusted for age and pill use). IGF-I levels were significantly suppressed by pill use in white women (301 vs 267 ng/ml; p = 0.0003); but not in black, Asian, or Indian-Pakistani women. IGF-I levels were higher among black women who used the pill than in black women who did not. Among pill users, the IGF-I level was positively associated the absence of the IGF1 19-repeat allele (r=0.29; p=0.00005), with the presence of the CYP3A4 variant allele (r=0.18; p=0.009), and with the presence of the AIB1 26-repeat allele (r=0.09; p=0.08). The cumulative number of these variant alleles was much higher among black than among white women (p<0.001), and IGF-I levels were strongly correlated to cumulative number of variant alleles (r=0.31; p=3x10-6). After adjusting for genotypes, ethnic group was no longer a significant predictor of the IGF-I level among pill users. The high incidence of premenopausal breast cancer among black women may be mediated through genetic modifiers of circulating IGF-I levels.

Program Nr: 255 from the 2000 ASHG Annual Meeting

Trends in cytogenetic evaluation of babies with congenital defects, 1968-1997. *S.A. Rasmussen[1], P.W. Yoon[1], A.S. Brown[1], E. Rhodenhiser[1], K.M. May[2]*. 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Department of Pediatrics, Division of Medical Genetics, Emory University, Atlanta, GA.

Major advances in cytogenetic techniques have occurred since identification of the human chromosome number in 1956. These advances include chromosome banding techniques, high-resolution banding, and fluorescence in situ hybridization (FISH) technology. The impact of these advances on referrals for cytogenetic analyses, however, has not been studied. We investigated trends in cytogenetic referrals and diagnoses of chromosome abnormalities using data from the Metropolitan Atlanta Congenital Defects Program (MACDP) for 1968 through 1997. MACDP is a population-based surveillance system that actively ascertains birth defects diagnosed in the first year of life among babies whose mothers reside in the metropolitan Atlanta area. This surveillance program uses multiple sources of ascertainment, including records from birth and referral hospitals and results from a regional cytogenetics laboratory. We found that the proportion of babies with birth defects on whom cytogenetic analyses had been performed increased substantially from 7.0% in 1968 to 22.3% in 1997. The proportion of babies with abnormal chromosome results also increased (3.8% in 1968 to 7.7% in 1997). The increase differed by maternal age, birth status (liveborn, stillborn, or induced abortion), type of birth defect, and type of chromosome abnormality diagnosed. This study demonstrates a marked trend toward more referrals for cytogenetic studies and diagnoses of chromosome abnormalities in a selected contemporary U.S. population. Understanding the impact of cytogenetic advances on referrals for cytogenetic analysis in babies with birth defects may give insight into how future laboratory advances may affect clinical care. In addition, the finding that cytogenetic studies were performed on a substantial proportion of babies in MACDP suggests that cytogenetic laboratories may be an excellent source for case ascertainment in birth defects surveillance programs.

Program Nr: 256 from the 2000 ASHG Annual Meeting

Applications, exhilarations, polymorphisms, and pitfalls: the use of telomere region-specific probes. *B.C. Ballif, C.D. Kashork, L.G. Shaffer.* Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The recent identification of a complete set of telomere region-specific FISH probes has facilitated the discovery of submicroscopic telomeric aberrations. We have used these probes to interrogate the telomeric regions of 174 patients. The utility of these FISH probes is evident in that 13/133 (9.8%) patients with mental retardation and/or dysmorphic features and an apparently normal karyotype had submicroscopic telomeric deletions or cryptic telomeric rearrangements identified. However, only 7/13 (54%) telomeric abnormalities were shown to potentially contribute to the phenotype since 6/13 (46%) patients inherited apparently benign telomeric variants from a normal parent. These include inheritance of deletion of 2q telomeric sequences and trisomy 13q telomeric sequences due to a derivative 1q. Interestingly, only 4/108 (3.7%) patients diagnosed with various major anomalies in addition to mental retardation (MR) and/or developmental delay (DD) had telomeric aberrations whereas 3/25 (12%) patients ascertained with only MR and/or DD showed telomeric abnormalities. Telomere region-specific probes were also used to investigate the mechanisms that generate and stabilize cytogenetically defined terminal deletions by analyzing chromosomes from 41 patients with 1p36 deletion syndrome. These results showed that 7/41 (17.1%) had a cryptic derivative 1p, 5/41 (12.2%) were interstitial deletions, and 29/41 (70.7%) were confirmed to be terminal deletions. Our data also shows that telomeric polymorphisms may be quite common (~ 4% of patients studied). As the limits of the technology are pushed further toward the ends of the chromosomes, more polymorphisms are likely to be identified. We caution that all telomeric abnormalities should be confirmed by testing parental samples to exclude the possibility of a benign familial polymorphism segregating in the affected child. In summary, the detection of cryptic telomeric aberrations in 3.7% - 12% of our study population, the elucidation of telomeric structure in cytogenetically defined terminal deletions, and the uncovering of telomeric polymorphisms illustrate the applications and pitfalls of telomere region-specific probes.

Program Nr: 257 from the 2000 ASHG Annual Meeting

A novel automated strategy for screening cryptic telomeric rearrangements in children with idiopathic mental retardation. *L. Colleaux[1], M. Rio[1], S. Heuertz[1], S. Moindrault[4], C. Turleau[1], MC. de Blois[3], O. Raoult[3], M. Prieur[3], S. Romana[3], F. Cornelis[4], M. Vekemans[3], A. Munnich[1,2]*. 1) INSERM U393, Hopital NECKER-ENFANTS MALADES, Paris, France; 2) Service de Genetique Medicale, Hopital NECKER-ENFANTS MALADES, Paris, France; 3) Service de Cytogenetique, Hopital NECKER-ENFANTS MALADES, Paris, France; 4) ECRAF-Universite Paris 7, GENOPOLE, Evry, France.

Cryptic unbalanced subtelomeric rearrangements are known to cause a significant proportion of idiopathic mental retardation in childhood. Because of the limited sensitivity of routine analyses, the cytogenetic detection of such rearrangements requires molecular techniques, namely FISH and comparative genomic hybridization (CGH). On the other hand, microsatellite markers are naturally occuring DNA polymorphisms that can be used to search for irregular allele inheritance and detection of deletions, duplications or uniparental disomies. Recently, a pilot study based on conventional autoradiographic procedures allowed to diagnose two deletions of telomeric regions in a series of 27 children with idiopathic mental retardation. In addition, several groups have demonstrated the accuracy of fluorescence-based technology for large scale genome mapping. We therefore developped a novel strategy based upon automated fluorescent genotyping to search for non mendelian segregation of telomeric microsatellites. A total of fifty six individuals belonging to forty six unrelated families were screened and eight abnormal patterns of segregation were detected : six rearrangements and two parental disomy. Our results confirm therefore the significant contribution of cryptic telomeric rearrangements to idiopathic mental retardation and demonstrate that fluorescent genotyping is a very sensitive and cost-effective method to detect such rearrangements. Finally, the microsatellite technique provides the unique opportunity to detect uniparental disomies.

In addition to its clinical relevance, this novel strategy will hopefully allow the delineation of new contigous gene syndromes and the identification of new imprinted regions.

Program Nr: 258 from the 2000 ASHG Annual Meeting

Identification of uniparental disomy in phenotypically-abnormal balanced carriers of Robertsonian translocations and isochromosomes. *S.A. Berend, C. McCaskill, L.G. Shaffer.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Most balanced carriers of acrocentric rearrangements, including Robertsonian translocations and isochromosomes, have a normal phenotype. Since rearrangements of the acrocentric chromosomes are associated with an increased risk for aneuploidy, and given the large number of reported cases of uniparental disomy (UPD) associated with an acrocentric rearrangement, carriers are presumed to be at risk for UPD. However, an accurate risk estimate for UPD in this population is lacking. The goal of this study was to provide an estimate of the risk for UPD associated with non-homologous Robertsonian translocations and homologous acrocentric rearrangements in balanced carriers who have an abnormal phenotype. Fifty individuals who carry a balanced acrocentric rearrangement, either a Robertsonian translocation or an isochromosome, and present with an abnormal phenotype were prospectively studied. The non-homologous rearrangements included thirty-two rob(13q14q), two rob(13q15q), one rob(13q22q), six rob(14q15q), four rob(14q21q), one rob(14q22q), one rob(15q22q) and one rob(21q22q). Two of 48 non-homologous Robertsonian translocations, both rob(14q15q), showed UPD (4%). Although, only two homologous acrocentric rearrangements [der(14q14q) and a der(15q15q)] were studied, both showed UPD (100%). Warburton (1991, Am J Hum Genet 49:995-1013) surveyed rearrangements in prenatal samples and estimated the risk for a serious congenital anomaly in de novo balanced carriers of Robertsonian translocations to be approximately 3.7%; perhaps a proportion of these had UPD. Given the relatively high risk for UPD in carriers of Robertsonian translocations and isochromosomes, UPD testing should be considered, especially for cases involving chromosomes 14 and 15 in which UPD is associated with adverse clinical outcomes. *de novo*.

Program Nr: 259 from the 2000 ASHG Annual Meeting

Non-Mendelian Segregation in Heterozygous Female Carriers of Robertsonian Translocations. *F. PardoManueldeVillena[1], C. Sapienza[1,2]*. 1) Fels Institute for Cancer Research, Temple University Medical School, Philadelphia, PA; 2) Department of Pathology, Temple University Medical School, Philadelphia, PA.

Robertsonian (Rob) translocations are the most common structural chromosome rearrangement in human. Although segregation of Rob chromosomes has been examined in many families, there is little consensus on whether inheritance in the balanced progeny conforms to Mendelian ratios. To address this question, we have compiled segregation data, by sex of parent, among 681 balanced offspring of Rob carriers from 121 informative families and from a large study on the risk of unbalanced offspring in carriers of chromosome rearrangements reported previously. Extreme care was taken to avoid all sources of ascertainment bias. Our analysis supports the following conclusions: 1) The segregation ratio is not independent of the sex of the carrier ($P<0.02$); 2) Significant departure from Mendelian inheritance ratios (transmission ratio distortion, TRD) is observed consistently only among the offspring of carrier females, and; 3) TRD is not dependent on the presence of a specific acrocentric chromosome in the rearrangement. Female carriers of Rob translocations transmit preferentially the Rob chromosome to their offspring ($P<0.001$). This, in turn, leads to an increased familial risk for aneuploidy because heterozygous female carriers are at high risk of forming aneuploid gametes due to adjacent segregation at the first meiotic division (MI). The sex-specific origin of TRD, the fact that the rearranged (mutant) chromosome is observed at significantly higher frequency than the acrocentric (normal) chromosomes and the similarities between these data and the non-Mendelian segregation of chromosomes in female Rob carriers in the mouse support the hypothesis that TRD in the human results from preferential segregation of chromosomes during MI (meiotic drive). In addition, this analysis provides evidence for polarity of the meiotic spindle and suggests a general mechanism by which homologous chromosomes may be distinguished from each other in their interaction with the spindle. Abstract sponsored by ASHG member Kenneth Morgan, McGill University.

Program Nr: 260 from the 2000 ASHG Annual Meeting

Molecular and cytogenetic delineation of marker chromosomes: Implications for phenotypic effects. *M.D. Graf[1], P.N. Mowrey[2], D.L. Van Dyke[3], M.J. Pettenati[4], E.E. Eichler[1], S. Schwartz[1].* 1) Dept of Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Diagnostic Genetics, Labcorp, RTP, NC; 3) Dept of Med Genetics, Henry Ford Hospital, Detroit, MI; 4) Division of Medical Genetics, Wake Forest Univ, Sch of Medicine, Winston-Salem, NC.

Over the past several years molecular techniques have aided in the delineation of marker chromosomes, however, little is yet known about the exact significance of many supernumerary chromosomes. This is extremely problematic, especially in cases of markers ascertained prenatally. To better understand the karyotype-phenotype relationship of these markers, we have utilized molecular cytogenetic technologies in many cases to describe these chromosomes. With the continual delineation of the human genome, a vast array of single copy and repetitive probes, have become available to evaluate the phenotypic significance of these chromosomes. In a collaborative effort we have collected over 275 markers, ascertained either prenatally or postnally. We have used both molecular techniques (using PCR with microsatellite markers) and molecular cytogenetics techniques (FISH with YACs, BACs and cosmids) to characterize the markers. Results from these studies show that: (1) the pattern of chromosomal involvement is significantly different between the postnatal and prenatal group (2) the chromosomal origin has an effect on the phenotypic outcome; (3) utilization of single copy DNA probes (BACs and cosmids) in over 40% of these cases have been effective in attempting to predict a normal or abnormal phenotype by showing whether specific genes are present; (4) a series of probes for repetitive DNA in the pericentromeric region of 15 different chromosomes have been developed that can be used in combination with single copy probes to help determine phenotype effectively in almost all prenatal cases. These studies highlight a benefit that the human genome project has had in the field of cytogenetics and provide a protocol for how markers should be analyzed. This methodology will allow for better phenotypic prediction of the marker chromosomes, and will especially help with genetic counseling in prenatal cases.

Program Nr: 261 from the 2000 ASHG Annual Meeting

Duplications of the Prader-Willi/Angelman Critical Region: An Association with Autism? *C.E. Browne[1], N.R. Dennis[2], P. Bolton[3], N.S. Thomas[1], P.A. Jacobs[1]*. 1) Wessex Regional Genetics Lab, Salisbury District Hospital, Wilts, United Kingdom; 2) Wessex Clinical Genetics Service, Princess Anne Hospital,Southampton,United Kingdom; 3) Section of Developmental Psychiatry, Douglas House, Cambridge,United Kingdom.

The phenotype associated with duplications of the proximal long arm of chromosome 15 (dup(15)) depends on both the parental origin and the presence or absence of the Prader-Willi/Angelman critical region (PWACR). Duplications with an additional maternal copy of the PWACR appear to be associated with mild to severe mental retardation and/or autism. Conversely, paternal PWACR dup(15)s appear to be without clinical effect. These conclusions, however, were based on a small number of cases. We present the results of the cytogenetic, molecular genetic and clinical analysis of 27 individuals from 10 families carrying a PWACR duplication. All but one of the duplications were cytogenetically visible. Molecular genetic analysis demonstrated 3 common breakpoints, 2 proximal and 1 distal, which coincide with those involved in PWACR deletions suggesting that the duplications are the reciprocal products of the deletions. The clinical findings suggest that paternally derived dup(15)s have little, if any, phenotypic effect, whilst the majority of maternal PWACR dup(15)s are associated with mild mental handicap. However, the degree of mental deficiency ranged from low-average intelligence to moderate or severe mental retardation, sometimes within the same family. Four probands from 3 families were diagnosed as having pervasive developmental disorder with only one meeting the criteria for classic autism. There was, therefore, very little evidence of the duplication segregating with autism spectrum disorder. The dup(15) phenotype is, in general, less severe than that of patients with triplications of chromosome 15 or markers of chromosome 15 origin indicating a possible dosage effect for the gene(s) involved. If, as proposed, additional copies of the maternal PWACR result in a clinical effect, the dup(15) phenotype would be associated with overexpression of a maternally imprinted gene within the 15q11-q13 region.

Program Nr: 262 from the 2000 ASHG Annual Meeting

Partial Duplications of the *APBA2*Gene in a Chromosome 15q Autism Region Corresponds with 15q Duplicon Structures. *E.L. Nurmi, M.K. Han, R.A. Kesterson, J.S. Sutcliffe.* Program in Human Genetics, Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN.

Intervals in proximal chromosome 15q have been implicated in autism spectrum phenotypes based on observations of chromosomal duplications and linkage disequilibrium in autism families. The amyloid precursor binding protein A2 (*APBA2*) gene was previously localized to the 15q11-q13 interval, proximal to a common "duplicon" utilized in the majority of interstitial deletions and duplications affecting this region. We have mapped *APBA2*distal to this duplicon, which contains *HERC2*and other sequences present in highly homologous, partially duplicated copies, at other duplicon structures within this complex chromosomal region. *APBA2*is one of several genes in this region which are attractive functional candidates for involvement in autism phenotypes. APBA2 is also known as MINT-2 for its ability to interact with the syntaxin-interacting protein munc-18, in a complex which functions in the control of neuronal synaptic vesicle trafficking and exocytosis; abnormal regulation of these processes could arguably lead to neurological dysfunction. Analysis of gene structure and expression reveals that the *APBA2*locus encodes multiple transcripts expressed at varying levels throughout the brain. *In situ*hybridization to mouse brain sections reveals a striking pattern of expression in cortical and limbic structures of particular relevance to autism. Genomic mapping and analysis of this gene reveal that DNA including a large exon with the initiating methionine and ~5-kb of upstream sequence is duplicated with highly homologous but distinct copies present at more distal sites consistent with duplicons utilized for large inverted duplicated marker chromosomes 15. Expression analysis reveals that at least one of these partial copies is transcriptionally inactive. These data provide further information on the complex nature of the 15q duplicons and will facilitate genetic analysis of *APBA2*in autism families.

Program Nr: 263 from the 2000 ASHG Annual Meeting

Phenotype/karyotype correlations and definition of a critical region in duplication 9p syndrome. *S.M. McGuire[1], C.A. Crowe[2], M.A. Micale[3], D.J. Wolff[4], J.L. Zackowski[5], L.A. Christ[1], S. Schwartz[1]*. 1) Case Western Reserve Univ, Cleveland, OH; 2) MetroHealth Medical Center, Cleveland, OH; 3) Medical College of Ohio, Toledo, OH; 4) Medical University of South Carolina, Charleston, SC; 5) Eastern Virginia Medical School, Norfolk, VA.

A duplication of the short arm of chromosome 9 is a well-documented cytogenetic and clinical entity. However, isolated duplications of 9p without concomitant deletions of other chromosomes make up a minority of cases, and only limited studies have utilized molecular techniques to characterize these isolated 9p duplications. Previous cytogenetic studies have implicated a region including 9p22 as the critical region leading to the duplication 9p syndrome phenotype, but this region has not been confirmed on a molecular level. We studied ten patients with isolated 9p duplications and three patients with 9p duplications and 9p deletions to make phenotype/karyotype correlations, further identify a critical region for duplication 9p syndrome, and understand the mechanism of formation of these duplications. We utilized molecular (PCR with microsatellite markers) and molecular cytogenetic (FISH with single copy YAC probes) analyses to define the breakpoints of each duplication. Results from these studies showed that the duplications ranged in size and orientation. Nine patients had intrachromosomal duplications, two patients had a supernumerary chromosome, and two patients had an isochromosome 9p. We can conclude that: 1) there is a consistent phenotype for duplication 9p syndrome despite varying sizes of the duplications; 2) a 3 Mb region between microsatellite markers D9S162 and D9S267 mapping to 9p22.2-p23 appears to be the critical region for the duplication 9p syndrome phenotype in our group of patients; and 3) a variety of mechanisms were suggested by these studies, including unequal crossing-over, telomeric exchanges as evidenced by interstitial telomeres and centromeric breakage as delineated by the presence of dicentric chromosomes. These studies are important because they allow for more specific phenotype/karyotype correlations, as well as a more precise delineation of the duplication 9p syndrome critical region.

Program Nr: 264 from the 2000 ASHG Annual Meeting

Maternal sex chromosome nondisjunction: evidence for X chromosome-specific risk factors. *N.S. Thomas[1], A.R. Collins[2], S.M. Ennis[2], A.J. Sharp[1], M. Durkie[1], T.J. Hassold[3], P.A. Jacobs[1,2].* 1) Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, Wiltshire, UK; 2) Human Genetics, Duthie Building, Southampton General Hospital, Southampton, UK; 3) Department of Genetics, Case Western Reserve University, Cleveland, USA.

The association between altered recombination and maternal nondisjunction is well documented, as reductions in recombination have been reported for maternal meiosis I (MI) errors involving chromosomes 15, 18, 21 and the sex chromosomes. However, the effects of recombination on MI errors involving women of different ages, or on maternal meiosis II (MII) errors, are less clear and may be chromosome specific. For example, in maternal MI errors, there is a direct correlation between exchange frequency and maternal age for trisomy 15, but not for trisomy 21; in maternal MII errors, increases in maternal age are associated with both trisomies 18 and 21, but recombination is normal for trisomy 18 and increased for trisomy 21. We therefore investigated maternal X chromosome nondisjunction, to determine whether the effects of recombination are unique to the X or similar to any of the autosomes thus far studied. We genotyped forty five 47,XXX females and ninety five 47,XXY males of maternal origin. Our results demonstrate that 49% arose during MI, 29% during MII, and that 16% were postzygotic events; a further 7% were meiotic but could not be assigned as either MI or MII because of recombination at the centromere. Among the MI cases, a majority (55%) had no detectable exchange, similar to previous studies of trisomy 21 but unlike those reported for trisomy 15. However, similar to trisomy 15 and unlike trisomy 21, we observed a significant increase in the mean maternal age of chiasmate MI errors (35.9 yrs) compared to achiasmate MI cases (30.8 yrs). In our studies of MII errors, recombination appeared normal and there was no obvious effect of maternal age, distinguishing our results from MII nondisjunction of either chromosomes 18 or 21. Thus surprisingly the risk factors associated with both MI and MII nondisjunction appear to be different for virtually every chromosome that has been adequately studied.

Program Nr: 265 from the 2000 ASHG Annual Meeting

Patient and professional views on ethics: a 21-clinic survey. *D.C. Wertz.* Social Science, Ethics & Law, Univ Massachusetts,Shriver Div, Waltham, MA.

We surveyed 718 patients at 12 genetics clinics and 1538 genetics providers. 476(59%) patients and 1084(70%) providers returned anonymous mail questionnaires. Patients were white(89%) working-class(70%) women(91%),Catholic background(44%), median family income $25-40,000, median 13y education. Patients and providers agreed on no access to genetic information without consent for insurers and employers; transmission of genetic information in adoption; full disclosure to patients; provider should/would perform or refer for PND for sex selection; DNA fingerprinting in serious crimes. Patients put greater emphasis than providers on individual autonomy: 59 v 36% said people were entitled to any service they request and can pay for; 69 v 56% said withholding any service was paternalistic; 80% v. 47% said PND should be provided on request or done for maternal anxiety only, without medical indications(75 v 30%). Fewer patients(41 v 82%) supported "rights not to know" information. Fewer patients would abort for 26 conditions, including trisomy 21(36 v 80%), achondroplasia(24 v 57%), XXY(22 v 49%), obesity(8 v 29%), HD(32 v 64%). Patients were more supportive of genetic testing: 40 v 10% said people at high risk should use PND; 81 v 38% said women with family histories should use PND; 66 v 44% said couples should know carrier status before marriage. More patients favored testing children for predisposition to Alzeimer(61 v 25%) or breast cancer(73 v 27%),telling blood relatives a diagnosis of HD without patient's consent(75 v 38%), spousal access to DNA without consent(74 v 36%), access for blood relatives(53 v 22%), or telling a husband about nonpaternity if he asks(74 v 36%). More patients(96 v. 26%) would tell a school system a diagnosis of XYY; fewer(20 v 62%) would preserve confidentiality of a bus driver with FH or thought workplace testing should be voluntary(51 v 94%). Results suggest that patients feel entitled to requested services, take a familial view of genetic information that includes relatives and spouses, support PND but are reluctant to abort and regard some third-party disclosure as benign. Extensive differences between patients and providers suggest a need for inclusion of patients in ethical policy discussions.

Program Nr: 266 from the 2000 ASHG Annual Meeting

A Model for Population-Based Governance of Genetics: Oklahoma Genetics Advisory Council (OGAC). *J.J. Mulvihill[1], N.J. Carpenter[2], M.A. Coffman[3], P. King[3], E.D. Rhoades[3].* 1) University of Oklahoma Health Sciences Center, Oklahoma City, OK; 2) H.A. Chapman Institute of Medical Genetics, Tulsa, OK; 3) Oklahoma State Department of Health, Oklahoma City, OK.

The Oklahoma State Department of Health established the Oklahoma Genetics Advisory Council in 1999. The objective was to anticipate and meet the ever rising expectations in genetics as applied to medicine and public health, as well as to accommodate new genetics programs at medical centers. The OGAC members, appointed by the State Commissioner of Health, are stakeholders in various facets of genetics services, education, and research: the 26 members include faculty at the two medical schools, private practitioners offering genetic services, representatives of state chapters of the American Academy of Pediatrics, the State Medical and Osteopathic Associations, the March of Dimes, the State Legislature, patient representatives, clergy, and the State Insurance Commissioner; 20 state health department professionals are ex-officio. By-laws are in place. At three meetings a year, OGAC reviews progress of six committees that meet between OGAC sessions: Newborn Screening Programs and Pediatrics; Birth Defects Registry and Prenatal Screening and Diagnosis; Education and Outreach; Adult Genetics; Public Health Genetics Policy, and Evaluation. Additional oversight is given by a small steering committee that meets monthly. Achievements to date are the award of a Federal grant to develop a state genetics plan, endorsement of three additional tests for newborn screening (CF, CAH, MCAD), and a plan for an educational program for all levels of professionals, in part based on a state genetics website under development and a toll-free number for genetics and teratogen information (1-877-OK-GENES). Our experience to date may help other jurisdictions develop strategies for shared development in genetics among academic medical centers, state health departments, and private health care systems, even as resources for genetic services decline, public demand increases, and public health opportunities emerge. (Supported by grants from Bureau of Maternal and Child Health and March of Dimes Oklahoma).

Program Nr: 267 from the 2000 ASHG Annual Meeting

The REVEAL Study: A New Model for Susceptibility Genotyping, Risk Assessment and Counseling for Alzheimer's Disease. *T.C. Brown[1], S.A. LaRusse[2], M. Barber[3], L.A. Farrer[1], L.A. Cupples[1], S. Post[3], A.D. Sadovnick[4], J.G. Davis[2], K.A. Quaid[5], P.J. Whitehouse[3], N. Relkin[2], R.C. Green[1]*. 1) Boston University School of Medicine, Boston, MA; 2) Weill Medical College of Cornell University, New York, NY; 3) Case Western Reserve University, Cleveland, OH; 4) University of British Columbia, Vancouver, BC CANADA; 5) Indiana University School of Medicine, Indianapolis, IN.

Genes and other markers for complex diseases are rapidly being identified and presymptomatic risk estimates are now possible. The REVEAL Study (Risk Evaluation and Education for Alzheimer's disease) is an NIH funded project (R.C. Green, PI) to examine the impact of providing risk assessment, including Apolipoprotein E (APOE) genotyping, for Alzheimer's disease (AD). REVEAL investigators are using the well-established genetic epidemiology of the disease, including risk attributable to different APOE polymorphisms, to develop risk profiles. Asymptomatic adult children of AD patients will be randomized into one arm where risk assessment is based upon age, gender and APOE genotype; or a control arm in which risk assessment is based only upon age and gender. Trained genetic counselors will communicate these risks (with APOE disclosure) and follow subjects carefully to determine the psychological and practical impact of this information. Outcome variables include measures of anxiety, depression and satisfaction with the risk assessment and counseling protocol, as well as real-world decisions to change retirement planning or insurance coverage. REVEAL will be the first study to explore susceptibility genotyping, risk assessment and counseling in an age-related degenerative disease. For this reason, preparation for REVEAL involved collection of pilot data, the establishment of internal and external review boards and the implementation of extensive safeguards, including a Certificate of Confidentiality. Results will inform policy debates and future guidelines about the advisability of such disclosures as the human genome is decoded and the scope of risk assessment expands. We present the development of this protocol as an example of research in this controversial area.

Program Nr: 268 from the 2000 ASHG Annual Meeting

Analysis of media reports on the discovery of breast and prostate cancer susceptibility genes. *B.A. Bernhardt, G. Geller, E. Tambor, E. Mountcastle-Shah, J.G. Mulle, N.A. Holtzman.* Genetics/Public Policy Studies, Johns Hopkins Medical Inst, Baltimore, MD.

Media coverage of disease-gene associations or genetic tests influences consumers' understanding of and interest in genetic technologies. As part of a study of media reporting of genetic discoveries, we developed a system for assessing the accuracy, balance and content of such reports in the print, radio and television media. Checklist items were generated by the investigators and by focus groups of 23 consumers. The checklist was then validated by asking 12 scientists and 16 journalists which items should be included. The final checklist included 36 items relating to description and credibility of the research, genetics and epidemiology of the disease-gene association, disease description, and implications of the discovery. We then analyzed 33 stories covering the cloning of the BRCA1 gene in 1994, and 17 stories covering linkage of a prostate cancer gene to chromosome 1 in 1996. We found that of the 26 checklist items applicable to the BRCA stories, an average of 49% were included and for the 22 prostate cancer items, 56% were included. Longer and print media stories included more items. Most stories mentioned: who did the research, where it was published, that the defects were inherited, prevalence of the cancer, portion of the disease attributed to the gene, and that a susceptibility test could be forthcoming. Only 15% of the BRCA stories mentioned treatment of breast cancer, while 75% mentioned prophylactic mastectomy. 65% of prostate gene stories mentioned treatment, while 6% mentioned prevention. 33% of BRCA and 30% of prostate gene stories indicated that the genes are associated with early-onset disease, and a minority mentioned to that the discovery pertains to high risk families. 42% of the BRCA and 18% of the prostate stories used at least one scientific term (usually mutation) without defining it. Although there were some false statements, most errors were ones of omission. Because of such deficiencies, people may believe that discovery of new genes will have immediate implications for broad segments of the population, including improved treatments or prevention.

Program Nr: 269 from the 2000 ASHG Annual Meeting

Automating the Family Cancer History. *L.S. Acheson[1,3], K.C. Stange[1,3], G.L. Wiesner[2,3]*. 1) Dept Family Medicine, Case Western Reserve Univ, Cleveland, OH; 2) Dept of Genetics, Case Western Reserve Univ., Cleveland, OH; 3) University Hospitals of Cleveland.

Obtaining the family cancer history is a time-consuming and labor-intensive component of cancer risk counseling. In the primary care setting, competing clinical demands have made obtaining a screening, 3-generation family cancer history impractical for the majority of patients. Thus, the potential for using family history information to recognize high-risk families, to tailor cancer screening and prevention efforts, and to identify patients for referral to genetic counselors and geneticists remains underutilized.

The Genetic Risk Easy Assessment Tool (GREAT)has been developed in conjunction with a commercial telecommunications and software company. It uses informatics technology to automate the collection and display of a detailed family cancer history. The GREAT allows patients anywhere, at any time convenient for them, to confidentially record their family cancer history by telephone or internet. Using a computer-administered interview whose sequence depends on the person's responses, the system quickly produces a pedigree that can be transmitted by fax or email to the clinician at the point of care. The interview also collects data on personal risk factors for cancer, such as smoking, reproductive history, ethnicity, colon polyps, and breast biopsy results, that will enable application of risk prediction models in future versions of the GREAT.

The GREAT pedigree specifically denotes the ages and genders of first, second, and third-degree relatives including half-siblings,twins, and first cousins, desplaying various types of cancer and the ages at diagnosis. The system has been used by 73 students and lay people who recorded their own family cancer histories. In addition, more than 200 test family histories have been recorded by the researchers and software developers. A pilot study to validate the family cancer history and pedigree produced via the GREAT, by comparing them with the "gold standard" interview by a genetic counselor, is in progress.

Program Nr: 270 from the 2000 ASHG Annual Meeting

Hereditary hemochromatosis testing for the C282Y, H63D, and S65C mutations in the *HFE* gene: the MSU experience. *L.M. Wolf, V. Leykam, M. Loomer, S.H. Elsea.* DNA Diagnostic Program, Dept of Pediatrics/Human Dev, Michigan State Univ, East Lansing, MI.

Hereditary hemochromatosis (HH) is the most common autosomal recessive disease in the Caucasian population. We have been offering *HFE* mutation testing since 1997 and have an educational program in place for patients and physicians. In 1999, a 609 samples were received for HH testing: 313 samples were received directly from patients, while 296 were received from physicians. Persons requesting direct testing must contact our program for counseling over the phone prior to receiving a testing kit by mail. The symptoms and inheritance of hemochromatosis, the nature of the three mutations, our 91% detection rate, the difference between information obtained from genetic testing and serum iron panels, and implications for other family members are all reviewed. While we require physician information with every sample, patients are given the option of withholding results from their physician. However, results are reported to both the patient and the doctor with regard to individuals identified who carry two mutations in the *HFE* gene. In 1999, 49% of results were reported to physicians only, 35% to patients only and 16% to both physicians and patients. Samples are accepted from a buchal brush or blood. Initial testing included the C282Y and H63D mutations; the S65C mutation was added in mid-1999. During 1999, our diagnostic population included: 15% C282Y homozygotes, 10% C282Y/H63D compound heterozygotes, 0.5% C282Y/S65C compound heterozygotes, 0.5% H63D/S65C compound heterozygotes, 1.5% H63D homozygotes, 20% C282Y heterozygotes, 18% H63D heterozygotes, and 1.5% S65C heterozygotes. These data are significantly different than what would be expected from general population screening, with 66% of the patients tested carrying at least one mutant allele. These results indicate that the general population is appropriately self-selecting for gene testing based on symptoms or family history. A bias in our sample is testing of family members once an affected individual is identified. We present data on *HFE* DNA testing spanning almost 4 years and including more than 2000 individuals.

Program Nr: 271 from the 2000 ASHG Annual Meeting

Sequence Analysis And Family Studies Suggest That CFTR-Dependent and -Independent Forms of Atypical Cystic Fibrosis Exist. *J.D. Groman, M.M. Egan, G.R. Cutting.* Cystic Fibrosis Foundation Genotyping Center and McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins School of Medicine, Baltimore, MD.

Cystic Fibrosis (CF) is an autosomal recessive disorder of exocrine impairment resulting in chronic obstructive sino-pulmonary disease, pancreatic insufficiency, male infertility, and high sweat electrolytes. Patients with atypical CF may present with one or a few of the above symptoms absent. The aim of this study is to determine the molecular genetic basis of atypical CF phenotypes. We analyzed 20 patients diagnosed with atypical CF. Ten patients carried a single mutation (Group 1), and ten carried no mutations (Group 2) after screening using a standard panel detecting 85% of known CFTR mutations. We sequenced all exonic sequence, bordering 15-30bp intronic sequence, and deep intronic mutations 3849+10kbC->T and 1811+1.6kbA->G of CFTR. Of the Group 1 patients, 9 of 10 had a second mutation identified via sequence analysis. Conversely, 1 of 10 Group 2 patients had both mutations identified by sequencing. Of the remaining Group 2 patients, one patient had one mutation identified, and 8 had no CFTR mutations. We chose to further study the families of two Group 2 patients negative for CF mutations. In Family 1, both daughters were diagnosed with atypical CF on the basis of chronic cough, recurrent lung infections, equivocal sweat Cl- levels; and an abnormal nasal potential difference measurement. In Family 2, 2 of 3 daughters were diagnosed with atypical CF based on Pseudomonas colonization, sinusitis, and pneumonia. Haplotype construction using RFLP and dinucleotide repeat markers within and flanking CFTR revealed that, in both families, affected siblings have not inherited identical CFTR alleles. The identification of multiple atypical CF patients lacking CFTR mutations, and the incomplete haplotype congruence in families with multiple affected siblings suggest that atypical CF is genetically heterogeneous, with CFTR-dependent and -independent forms.

Program Nr: 272 from the 2000 ASHG Annual Meeting

Complete scanning of the CFTR gene by D-HPLC. *C. Le Marechal[1], M.P. Audrezet[2], O. Raguenes[2], I. Quere[2], S. Langonne[2], C. Ferec[1,2]*. 1) EFS Bretagne, Brest, France; 2) Dept molecular Genetics, Hospital, University, Brest, france.

More than 900 different point mutations and more than 300 polymorphisms have been reported by the Cystic Fibrosis Genetic Analysis Consortium in the CFTR gene (http://www.genet.sickkids.on.ca). In this study we have scanned the 27 exons of the CFTR gene by Denaturing-HPLC (ion-pair reverse-phase high pressure liquid chromatography). After PCR, this technique divides heteroduplexes from homoduplexes formed by the mixing of wild type and mutated sequences. 30 couples of primers were designed and 467 different nucleotide changes through the gene have been looked for. D-HPLC has detected all the 404 mutations previously identified using DGGE followed by direct sequencing and 63 additional novel mutations or polymorphisms. Samples were collected among the following patient cohort : 1552 cystic fibrosis, 253 congenital bilateral absence of vas deferent and a control population. This new technique for the screening of DNA sequence variation has been found very efficient and rapid (100% of our CFTR alleles are so far identified and a complete CFTR gene scanning can be carried out in less than one week). D-HPLC can be automated and used for both research and routine (time saving and analysis cost reduction). It will allow decreasing the rate of untyped CFTR alleles in the different populations of interest. This is a major breakthrough in the field of molecular genetic analysis for cystic fibrosis, with important implications for prenatal diagnosis, cascade screening as well as genotype-phenotype correlation.

Program Nr: 273 from the 2000 ASHG Annual Meeting

A protocol for IRB oversight of clinically indicated research testing. *R.J. Hopkin[1], K. Huelsman[1], J. Johnson[1], T. Korfhagen[2]*. 1) Dept Human Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Institutional Review Board, Children's Hosp Medical Ctr, Cincinnati, OH.

At times it is difficult to distinguish between clinical and research genetic testing. Families evaluated in the genetics clinic frequently request testing to clarify a diagnosis. However, there are many genetic conditions for which testing is performed only by a research laboratory. Guidelines published by the federal government state that each institution participating in a research project must have its own institutional review, and that each federally funded project must be individually reviewed by each participating center. This specifically includes subject recruitment. The result is that IRB's have been flooded with protocols for research testing much of which is requested for clinical indications. To reduce the burden on the IRB and clinicians we developed a standard protocol and consent forms for clinically indicated research based genetic testing. The protocol contains a clear functional definition of research testing requiring institutional review. There is flexibility to include anonymous and identifiable patients. Procedures are included to protect patient confidentiality. All communication is through the genetics clinic. A logbook provides documentation for an annual report to the IRB. This oversight on an institutional level encourages tracking and review of testing submitted to outside research laboratories. It also provides mechanisms for grievances or unanticipated outcomes to be addressed. The protocol is approved for use only by genetics professionals. It requires provision of pretest counseling, and provides for optional follow-up counseling with result disclosure. It also addresses which portions of the evaluation and testing process can be billed to the patient or patient's insurance and which require other sources of funding. We propose this as an efficient working model for consideration by other institutions wishing to address these issues.

Program Nr: 274 from the 2000 ASHG Annual Meeting

Genetic proficiency testing in diagnostic laboratories - quality control is the message. *E. Dequeker, J. Cassiman.* Center for Human Genetics, KU Leuven, Leuven, Belgium.

To evaluate the quality of genetic testing for cystic fibrosis (CF), European Quality assessment schemes have been set up since 1996 (European concerted action for cystic fibrosis BMH4-CT96-0492, European CF Thematic Network QLK3-CT1999-00241). Six purified DNA samples were sent annually to respectively 136, 145, 159 and 173 laboratories with the request to test for the presence of CFTR mutations using their routine protocols. In the first three schemes only the genotyping results were evaluated together with the raw data. Since 1999, the design of the QA scheme included also an evaluation of the interpretation of the data in the report as it is normally being sent to the clinicians. Over the years the quality of genetic testing for CF improved but the error rate (1996: 35%, 1997: 24%, 1998: 21%, 1999: 9%) remained high. Moreover the results challenge the notion that only a small number of laboratories is responsible for the mistakes in the consecutive schemes. Apart from incorrect genotyping results due to technical reasons and misinterpretation of (technical correct) results, administrative mistakes were also made. With regard to the interpretation of the data in the written reports, the scheme of 1999 demonstrated that the way of reporting laboratory results varies considerably between the different laboratories. Unfortunately, 31% of the reports contained errors. 25% of these reports contained administrative errors, another 25% of the errors were due to mistakes in risk calculation which were included. However, in half of these reports, the error was due to a wrong interpretation of the results or the way of reporting. We conclude that genetic proficiency testing contribute to an overall improvement of the quality of genetic testing. However, continued efforts will be needed to further improve the genetic services provided to the community. To this end recommendations for the quality improvement of genetic testing in CF were formulated and reviewed by an expert panel (E. Dequeker et al Eur. J. Hum. Genet in press).

Program Nr: 275 from the 2000 ASHG Annual Meeting

The Molecular Basis of Human 3-Methylcrotonyl-CoA Carboxylase (MCC) Deficiency. *M.R. Baumgartner[1,4], S. Almashanu[2], R.N. Cole[3], T. Suormala[4], E.R. Baumgartner[4], D. Valle[1,2].* 1) Dept. of Pediatrics; 2) Howard Hughes Medical Institute; 3) Dept. of Biological Chemistry,Johns Hopkins University, Baltimore, MD; 4) University Children's Hospital, Basel, Switzerland.

Isolated, biotin-resistant MCC-deficiency is a rare disorder of leucine catabolism inherited as an autosomal recessive trait. The clinical presentation is extremely variable. Most patients present with an acute metabolic decompensation before 3 years of age. MCC is the last of 4 biotin-dependent human carboxylases to be cloned. MCC is composed of 2 nonidentical subunits, an a subunit which covalently binds biotin and a smaller b subunit. Here, we report molecular cloning of a and b subunits of human MCC. Using *A. thaliana* MCC a and b sequence we identified EST candidates and assembled full length mouse and human MCC a and b cDNAs. The human candidates predict an MCC a of 725 amino acids and an MCC b of 563 amino acids with 46% and 56% identity to the MCC a and b of *A. thaliana*, respectively. MCC b has 17 exons and maps to chromosome 5q12. MCC a maps to chromosome 3. We confirmed the identity of MCC a and b by purifying it from mouse liver using streptavidin Dynabeads followed by sequencing tryptic fragments using MALDI and electrospray tandem mass spectrometry. Previous studies in 13 probands with MCC-deficiency revealed 2 complementation groups (CG). Biochemical studies suggest that CG2 has a primary defect in MCC a. We amplified patient MCC a and b cDNA from fibroblast RNA and sequenced the product directly. Sofar we identified 5 mutant MCC a alleles in CG2 patients and 9 mutant MCC b alleles in CG1 patients including missense, frameshift and splice mutations. All mutations were confirmed by amplifying and sequencing genomic DNA. Our results confirm the identification of the human MCC a and b genes and the correct assignment of CG2 to a primary defect in MCC a. Functional testing of missense mutations and further characterization of the human MCC a and b subunits will provide the molecular basis to search for a correlation of genotype with the phenotypic variation in our patient population and will lead to a better understanding of the variable expression of this disorder.

Program Nr: 276 from the 2000 ASHG Annual Meeting

Isolated 2-methylbutyrylglycinuria caused by short-branched-chain acyl-CoA dehydrogenase deficiency: Identification of a new enzyme defect, resolution of its molecular basis and evidence for separate acyl-CoA dehydrogenases in isoleucine and valine metabolism. *B.S. Andresen[1,2], E. Christensen[3], T.J. Corydon[1], P. Bross[2], B. Pilgaard[4], R. Wanders[5], J. Ruiter[5], H. Simonsen[3], V. Winter[2], I. Knudsen[2], L.D. Schroeder[1,2], N. Gregersen[2], F. Skovby[3]*. 1) Inst Human Genetics , Aarhus University, Denmark; 2) Res. Unit f. Molec. Medicine, Aarhus University Hospital, Aarhus, Denmark; 3) Clinical Genetics, Rigshospitalet, Copenhagen, Denmark; 4) Pediatrics, Roskilde Amtssygehus, Denmark; 5) Emma Children's Hospital, AMC, University of Amsterdam, The Netherlands.

Acyl-CoA dehydrogenase (ACAD) defects in isoleucine and valine catabolism have been proposed in clinically diverse patients with an abnormal pattern of metabolites in their urine, but they have not been proven enzymatically or genetically, and it is unknown if one or two ACADs are involved. We investigated a patient with isolated 2-methylbutyrylglycinuria, suggestive of a defect in isoleucine catabolism. Sequence analysis of candidate ACADs revealed heterozygosity for the common SCAD A625 variant allele, no mutations in ACAD-8, but a 100 bp deletion in SBCAD cDNA from the patient. Identification of the SBCAD gene structure (11 exons, >20 kb.) enabeled analysis of genomic DNA. This showed that the deletion was caused by skipping of exon 10 due to homozygosity for a 1228G>A mutation in the patient. This mutation was not present in 118 control chromosomes. In vitro transcription/translation experiments and overexpression in COS cells confirmed the disease-causing nature of the mutant SBCAD protein, showed that ACAD-8 is an isobutyryl-CoA dehydrogenase and that both wild-type proteins are imported into and form tetramers inside mitochondria. Enzyme assay of patient cells confirmed the defect. MS/MS analysis of the new-born blood spot from the patient showed a normal acyl-carnitine profile. This and demonstration of homozygosity for 1228G>A in the asymptomatic mother may indicate that this enzyme defect is underdiagnosed. In conclusion, we report the first mutation in the SBCAD gene, show that it results in an isolated defect in isoleucine catabolism, and indicate that ACAD-8 functions in valine catabolism.

Program Nr: 277 from the 2000 ASHG Annual Meeting

Identification of isobutyryl-CoA dehydrogenase and its deficiency in humans. *J. Vockley[1], T.V. Nguyen[1], S. Ghisla[2], A.-W. Mohsen[1], D. Roe[3], C.R. Roe[3]*. 1) Dept Medical Genetics, Mayo Clinic, Rochester, MN; 2) Faculty of Biology, Univ. Konstanz, Konstanz, Germany; 3) Institute of Metabolic Disease, Baylor Univ., Dallas, TX.

The acyl-CoA dehydrogenases (ACDs) are a family of related enzymes which catalyze the a,b-dehydrogenation of acyl-CoA esters, transferring electrons to electron transferring flavoprotein. Two homologues active in branched chain amino acid metabolism have previously been identified. One of these, short-branched chain acyl-CoA dehydrogenase, has been postulated to catalyze reactions in both the valine and isoleucine catabolic pathways, but the human enzyme utilizes isobutyryl-CoA as substrate poorly. Recently, a new gene of unproven function has been identified and postulated to be an ACD on the basis of sequence homology *(ACD8)*. We have used expression in *E. coli* to produce and characterize the substrate specificity of this enzyme. Testing with different acyl-CoA substrates shows that the expressed enzyme has maximal activity with isobutyryl-CoA (23% activity with S-2 methylbutyryl-CoA as substrate as compared to isobutyryl-CoA) and no activity with butyryl-, valeryl-, or isovaleryl-CoA. A single patient has previously been described in whom fibroblast metabolic loading studies revealed an increase in accumulation of isobutyrylcarnitine, and a decreased oxidation of labeled valine. Metabolism of labeled isoleucine was normal. Amplified *ACD8* cDNA made from fibroblast mRNA from this patient is homozygous for a single nucleotide change (G905A) compared to mRNA from control cells. This predicts an R302N alteration in the precursor protein. Molecular modeling studies indicate that this mutation is located at the monomer interface of the holoenzyme tetramer, and predicts that the aberrant protein will be unstable. Expression of this mutant enzyme in our *E. coli* system leads to the production of an aggregated, inactive protein. Our findings unequivocally demonstrate the presence of a new ACD in humans specific to valine catabolism (isobutyryl-CoA dehydrogenase), along with the first definitive identification of a patient deficient in this enzyme.

Program Nr: 278 from the 2000 ASHG Annual Meeting

Characterizing the organization of the “liver-type” carnitine palmitoyltransferase 1 (L-CPT1) gene helps unravelling the molecular basis of L-CPT1 deficiency in human. *S. GOBIN[1], JP. BONNEFONT[1], C. PRIP-BUUS[2], C. MUNIER[1], M. FERREC[1], A. AVON[1], F. DEMAUGRE[1], JM. SAUDUBRAY[1], N. KADHOM[1], W. WILCOX[3], S. CEDERBAUM[4], A. GREEN[5], G. GRAY[5], P. MCKIERNAN[5], RJ. POLLITT[5], L. THUILLIER[1]*. 1) INSERM U393 and Genetic Biochemistry Dept, Hopital Necker, Paris and; 2) CNRS UPR1524, Meudon, France; 3) Dept of Pediatrics, Cedars-Sinai Medical Center and; 4) Mental Retardation Research Center, Los Angeles, CA; 5) Dept of Clinical Chemistry, Birmingham Childrens Hospital / Neonatal Screening Laboratory, The Children's Hospital, Sheffield, UK.

L-CPT1 deficiency is an autosomal recessive disorder of mitochondrial fatty acid oxidation causing recurrent attacks of severe hypoglycemia triggered by fasting. A total of 15 families have been reported so far, but only 2 CPT1 mutations have been hitherto described, namely the D454G (Iljst, J. Clin. Invest. 1998;102:527) and G710E mutations (Abadi, 49 ASHG meeting,1999). The sequence of the L-CPT1 cDNA is known but the organization of the gene has not been described yet. Detection of the disease-causing mutations has an important impact for both genetic counselling and the elucidation of the structure-function relationships. We have characterized the organization of the L-CPT1 gene by taking advantage both of the homology of the M-CPT1 (“muscle-type CPT1”) and L-CPT1 genes, and of the knowledge of the M-CPT1 gene structure. The L-CPT1 gene is made up of at least 19 exons similar in size to the M-CPT1 ones, while introns are markedly larger than those of M-CPT1. Furthermore, 3 potential promoter regions (- 4 kb to - 2kb upstream from the initiator ATG) and a putative polyA signal have ben identified (1 kb downstream from the stop codon). These data allowed us to identify a novel homozygous nonsense mutation in exon 4, two mutations in introns 13 and 15 in a compound heterozygous patient and a large homozygous deletion of the L-CPT1 gene. Thus, identification of the L-CPT1 gene organization enables both an easy molecular screening and the dissection of the regulatory mechanisms of the L-CPT1 gene expression.

Program Nr: 279 from the 2000 ASHG Annual Meeting

The clinical spectrum of cytochrome c deficiency in Leigh syndrome patients with and without mutations in the SURF1 gene. *H.H.M. Dahl, W.M. Hutchison, A. Dasvarma, K. Reed, D.M. Kirby, A. Boneh, J. Christodoulou, M.L. Freckmann, B. Wilcken, J.J. McGill, J.L. Fletcher, J. Van Hove, D.R. Thorburn.* Murdoch Childrens Research Inst, Royal Children's Hosp, Melbourne, Australia.

Leigh syndrome is one of the most common disorders of mitochondrial energy generation presenting in childhood. The classic clinical features include progressive neurological disease with motor and intellectual developmental regression, signs and symptoms of brainstem or basal ganglia disease, lactic acidosis, and characteristic neuropathological features on cerebral imaging or at postmortem. Leigh syndrome can be caused by a number of enzyme defects including cytochrome c oxidase (COX) deficiency. Mutations in the SURF1 gene were recently shown to be a common cause of COX-deficient Leigh syndrome. We have evaluated 20 patients with isolated COX-deficient Leigh syndrome, and found SURF1 mutations in 13, all of Anglo-Celtic or European ancestry. A common deletion/insertion mutation in exon 4 represented 16 of the 26 mutant alleles, and cosegregated with two silent intragenic polymorphisms. This haplotype is present in less than 10% of the population, suggesting that the common SURF1 mutation originally arose on this haplotype. Seven patients of Lebanese ancestry had no mutations detected in the SURF1 gene and were shown by fibroblast cell fusion studies to represent a different complementation group. COX-deficient Leigh syndrome has been regarded as a systemic disease with little or no difference in COX activity between different tissues, but interestingly the two patient groups had striking differences in clinical presentation. The Lebanese patients had severe global developmental delay and seizures whereas those with SURF1 mutations tended to have a later onset with marked sparing of cognitive function, but suffered severe ataxia, renal tubular acidosis, and hypertrichosis. These differences suggest that there must either be tissue-specific differences in expression of the mutated genes or different pathogenic mechanisms involved.

Program Nr: 280 from the 2000 ASHG Annual Meeting

NDUFV1 mutations in mitochondrial complex I deficiency. *P. Benit, P. de Lonlay-Debeney, A. Munnich, P. Rustin, A. Rotig.* Dept Genetics, INSERM U393, Hosp Necker, Paris, France.

NADH:ubiquinone oxidoreductase (complex I) is the largest complex of the mitochondrial respiratory chain and this complex accounts for most cases of respiratory chain deficiency in human. Only seven mitochondrial DNA genes but more than 35 nuclear genes encode complex I. Among them, the NDUFV1 gene encodes the 51 kDa subunit which contains the NADH-, FMN- and Fe-S-binding sites. In an attempt to elucidate the molecular bases of the respiratory chain disorders, we studied the NDUFV1 gene in a series of 36 patients with isolated complex I deficiency, after having excluded mitochondrial DNA mutations. The full length NDUFV1 mRNA extracted from cultured skin fibroblasts was reverse transcribed and tested by Denaturing High Performance Liquid Chromatography (DHPLC). Abnormal fragments were then directly sequenced. Six novel missense mutations were identified in three patients presenting with Leigh syndrome. All patients, born to non-consanguineous parents, were compound heterozygotes and the parents were heterozygous for the corresponding mutations. These mutations involved highly conserved amino acids. Most interestingly, 3/6 mutations altered the FMN-binding site of the 51 kDa subunit in two patients. Considering that the patients had normal respiratory chain activities in cultured fibroblasts, we hypothesize that the high concentration of FMN in the culture medium might have restored a normal complex I activity. These results suggest that screening for NDUFV1 mutations is of great interest in patients with complex I deficiency, (even when normal respiratory chain enzyme activities in cultured fibroblasts is observed). In addition, they should prompt us to systematically put complex I deficient patients on a high riboflavin diet.

Program Nr: 281 from the 2000 ASHG Annual Meeting

Mitochondrial DNA repair in Saccharomyces cerevisiae. *S.L. Donahue, C. Campbell.* Dept Pharmacology, Univ Minnesota Medical Sch, Minneapolis, MN.

Mutations within human mitochondrial DNA are associated with a number of human disorders, and may play a causal role in the aging process. However, the mechanism through which these mutations arise is obscure. We are using the yeast *Saccharomyces cerevisiae* as a model system to gain insight into basic molecular mechanisms of mitochondrial DNA mutagenesis and DNA repair. A key component of DNA replication and repair processes is DNA ligase. In *S. cerevisiae*, there are two known DNA ligase proteins, Cdc9p and Dnl4p. The Cdc9p DNA ligase is known to be targeted to the mitochondria. However, the role that this molecule plays in mitochondrial DNA dynamics is ill-defined. It was also unclear whether Dnl4p or other, unknown DNA ligase proteins may be present in the yeast mitochondria. To address these issues, a biochemical and genetic analysis of mitochondrial DNA ligase function was performed. We found that, in contrast to Cdc9p, the Dnl4p ligase was not essential for maintenance of yeast mitochondria. Analysis of mitochondrial protein extracts derived from strains lacking mitochondrial Cdc9p provided no evidence for any other DNA ligase activity in yeast mitochondria. Inactivation of mitochondrial Cdc9p function led to a rapid decline in cellular mitochondrial DNA content in both dividing and stationary cultures. A novel strategy employing an *Eco* R I endonuclease fused to a mitochondrial targeting sequence was used to create DNA double-strand breaks within the yeast mitochondrial genome. Transient expression of the *Eco* R I enzyme in the mitochondria led to massive mitochondrial DNA double-strand breaks. Although wild-type and dnl4-null yeast were able to rapidly recover from this mitochondrial DNA damage, clones deficient in mitochondrial-targeted Cdc9p were not. Our results indicate that yeast apparently rely upon a single DNA ligase to carry out mitochondrial DNA replication as well as to repair spontaneous and induced mitochondrial DNA damage. We are currently performing a structure-function analysis of the mitochondrial Cdc9p to identify domains within this protein that are required for these replication and repair processes.

Program Nr: 282 from the 2000 ASHG Annual Meeting

Fasting fuel homeostasis triggered by defective phytanic and pristanic acids metabolism in the 70kDa peroxisomal membrane protein (PMP70) deficient mice. *G. Jimenez-Sanchez[1], K.J. Hebron[1], I. Silva-Zolezzi[1], S. Mihalik[2], P. Watkins[2], M. Espeel[3], A. Moser[2], G. Thomas[2], F. Röels[3], D. Valle[1]*. 1) HHMI and Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Kennedy Krieger Institute, Baltimore, MD; 3) Dept. Human Anatomy & Embryology, University of Gent, Belgium.

ATP-binding cassette (ABC) transporters are members of a superfamily of membrane proteins. There are 4 known human peroxisome half ABC transporters: PMP70, ALDP, ALDP-related and PMP70-related. Mutations in ALD are responsible for X-ALD. The function of the other 3 is unknown. To better understand the function of PMP70 and its relationship to human disease, we produced PMP70-/- mice by standard methods. PMP70-/- fibroblasts contain peroxisomes with normal amounts of PTS1 and PTS2 targeted matrix proteins. Liver peroxisomes had a significantly higher cellular volume density (mean d-circle: 0.753mm vs. 0.452mm in controls). We found a dramatic (10X) reduction in hepatic glycogen (HG) of the PMP70-/- mice in the fed state. Glycogen synthase mRNA and activity levels are similar to control thus the mechanism of this may relate to HG utilization. PMP70-/- fibroblasts and liver showed a defect in phytanic and pristanic acids oxidation (~50%). M oreover, PMP70-/- animals on a phytol supplemented diet showed a 10X accumulation of plasma phytanic and pristanic acids. Urinary organic acid analysis showed a striking medium chain dicarboxylic aciduria (DCA) that increases during fasting. PMP70-/- mice have defective nonshivering thermogenesis (DNST) with a drop in body temperature to <15°C after 3 hours at 4°C (Controls 34±2°C). These results suggest impaired mitochondrial b-oxidation. In addition, PMP70-/- mice have an increased expression of peroxisomal acyl CoA oxidase, mitochondrial MCAD, and CYP 4A3, all encoded by genes regulated by PPARa. We propose that PMP70 is essential for peroxisomal branched chain fatty acid metabolism, and that lack of PMP70 causes accumulation of these metabolites which in turn activate PPARa, leading to peroxisome enlargement and inappropriate activation of fasting fuel homeostasis, resulting in reduced HG, DNST, and DCA.

Program Nr: 283 from the 2000 ASHG Annual Meeting

Glycerol Kinase (GK) Deficiency: Expression of GK Mutations in Cos-7 Cells Confirms that the Phenotype of this Simple Mendelian Disorder is a Complex Trait. *K.M. Dipple, K. Nagano, J. Havens, J. Chang, Y.H. Zhang, B.L. Huang, R. Keynigshteyn, E.R.B. McCabe.* Dept Pediatrics, Univ California Los Angeles, Los Angeles, CA.

Isolated X-linked glycerol kinase deficiency (GKD) may be either symptomatic (episodic metabolic and central nervous system decompensation) or asymptomatic (only pseudo-hypertriglyceridemia). We have previously shown that one symptomatic patient with GKD is indistinguishable from asymptomatic individuals by enzyme activity measurements in cell lines and positions of mutations in a GK molecular model. The purpose of this study was to examine the consequences of GK mutations transfected into Cos-7 cells. We carried out site-directed mutagenesis of human GK cDNA cloned into a pCAGGs expression vector, producing mutations observed in two symptomatic patients (R413X, R405Q) and six asymptomatic individuals (D198G, N288D, A305V, M428T, Q438R, D440V). The GK activity was measured by a radiochemical assay. The nonsense mutation (R413X) from one symptomatic patient showed no activity, but the symptomatic individual with the missense mutation (R405Q) had significant residual activity (1.7% of control), which was greater than the activities of the three asymptomatic individuals whose mutations mapped closest to it in our structural model (0-0.7%) and higher than all but one (N288D: 4.8%) of the asymptomatic individuals' GK activities. The relative order of the mutant activities for the constructs in Cos-7 cells differed from those we had previously measured in the individuals' cell lines. We conclude that the observations in transfected Cos-7 cells confirm our previous observation that there is no genotype-phenotype correlation among patients with GKD. The differences observed between the mutant GK activities in lymphoblastoid cell lines and Cos-7 cells are consistent with our proposal that modifier genes, which would differ in expression in these cell types, influence phenotype, including the biochemical phenotype. These observations support our proposal that the clinical phenotype of this simple Mendelian disorder is a complex trait.

Program Nr: 284 from the 2000 ASHG Annual Meeting

Structure-Function Analyses of A Common Mutation in Blacks with Transferase-Deficient Galactosemia. *L.J. Elsas, K. Lai.* Dept Pediatrics, Div Medical Genetics, Emory Univ, Atlanta, GA.

We previously identified a missense mutation at amino acid 135 of galactose-1-phosphate uridyltransferase (GALT) in which a leucine (TTG) was substituted for a serine (TCG), (S135L). This mutation resulted in no erythrocyte or lymphoblast GALT activity or protein, 10% GALT in leukocytes, but normal total body ^{13}C-galactose oxidation to $^{13}CO_2$ by homozygotes (S135L/S135L). We modeled this substitution against the X-ray crystal structure of the homologous *E. coli* GalT and analyzed purified human GALT proteins with S135, A135, C135, L135, T135, H135 and a double substitution S132-H135.

	110	111	112	113	114	115	116	117	118
E.coli	C	F	S	P	D	H	S	K	T
Human	C	F	H	P	W	S	D	V	T
	130	131	132	133	134	135	136	137	138

These 7 GALT proteins were expressed in *E. coli*, purified and used to quantitiate GALT's double displacement reactions. We found normal glucose-1-phosphate production (first displacement) but a markedly impaired second displacement reaction for all substitutions, except T135-GALT. Activities for S135, A135, C135, H135, L135, S132-H135, and T135 GALTs were 100%, 4.7%, 3.0%, 0.2%, 0.7%, 1.0%, and 35.4% respectively. There was no difference in the thermolability of S135- and L135-GALT. We conclude that hGALT requires an hydroxy group at amino acid 135 to enable the catalysis of galactose-1-phosphate to UDP-galactose, that GALT enzyme abundance in different organs is not due to the thermolability of L135-GALT, and that organ-specific regulation of GALT gene expression and protein biostability may account for the disparities of total enzyme activity in different organs of patients with the S135L mutation.

Program Nr: 285 from the 2000 ASHG Annual Meeting

A deletion encompassing *Zic3* in Bent tail, a mouse model for X-linked neural tube defects. *B. Franke[1], R. Klootwijk[1], C.E.E.M. van der Zee[2], R.T. de Boer[3], H.W. van Straaten[4], W. Wilms[5], F.A. Hol[1], E.C.M. Mariman[1]*. 1) Department of Human Genetics; 2) Department of Cellbiology; 3) Department of Anatomy, UMC Nijmegen, Nijmegen; 4) Department of Anatomy and Embryology, University of Maastricht, Maastricht; 5) Central Animal Laboratory, UMC Nijmegen, Nijmegen, The Netherlands.

Neural tube defects (NTD) are congenital malformations with an incidence of 1-2 per 1000 births. In NTD the neural tube fails to fuse completely during embryonal development leading e.g. to anencephaly or spina bifida. NTD is a multifactorial disease, being caused by the combined effect of environmental and genetic factors. To date over 60 mouse models have been described that enable the study of the genetic factors involved in NTD. Bent tail (Bn) is a mouse model for X-linked NTD. Bn mice are characterized by a kinked tail. In Bn embryos we have observed abnormalities including exencephaly, rotation defects, omphalocele and occasionally orofacial schisis. The exencephaly is consistently caused by a fusion defect of the neural tube in part of the midbrain and the hindbrain region. *Bn* maps to the proximal part of the X chromosome. In the Juli issue of *Human Molecular Genetics* we report the gene defect of Bn, a deletion encompassing the *Zic3* gene. *Zic3* is a homolog of the *Drosophila* segmentation gene *odd-paired* encoding a zinc finger transcription factor expressed in murine neuroectoderm during neurulation. Data on *Zic3* expression and function suggest that deletion of *Zic3* is the main cause of the Bent tail phenotype in the mouse. Recently, endclone analysis of YACs in the region of *Zic3* has shown that the Bn deletion spans less than 890 kb. Currently we are further characterizing the deleted area and we will show data on at least one additional gene that is deleted in Bn. In man mutations in *ZIC3* have already been shown to lead to situs abnormalities, occasionally associated with NTD. To assess the relevance of *ZIC3* as a risk factor for human NTD, mutation studies have been performed in a panel of sporadic as well as familial cases of NTD including a large ijslandic pedigree.

Program Nr: 286 from the 2000 ASHG Annual Meeting

The X-linked Mouse Mutation Bent Tail is Associated with a Deletion of the Zic3 Locus. *T.L. Carrel[1], S.M. Purandare[2], W. Harrison[3], F. Elder[3], T. Fox[4], B. Casey[2], G.E. Herman[1].* 1) Department of Pediatrics, The Ohio State University, Columbus, OH; 2) Department of Pathology, Baylor College of Medicine, Houston, TX; 3) Department of Pathology, University of Texas Health Science Center, Houton, TX; 4) Department of Pathology, The Ohio State University, Columbus, OH.

The frequency of neural tube defects is second only to congenital heart disease in regard to congential malformations, with an estimated incidence of between 1-9/1000 total births. Several mouse mutants exist that are presumed models of human neural tube defects. Bent tail (*Bn*) is a spontaneous, semi-dominant, mutation on the mouse X chromosome that produces tail deformities and occassionally open neural tube defects. These defects are observed in homozygous females and hemizygous males as short, kinked tails. Heterozygous females show variable expression due to X inactivation. A backcross between inbred *Bn* and C3H mice was established, and PCR-based polymorphic microsatellites have been utilized to map the *Bn* locus as a prelude to isolation of the gene. Analysis of 292 normal male and affected male and female progeny places the *Bn* mutation in a 2.4 cM region between *DXmit166* and *DXmit140*. Refined genetic and physical mapping of the *Bn* critical region demonstrated that the mutation was associated with a <170 kb submicroscopic deletion that includes the anonymous microsatellite marker *DXMit208* as well as the the entire *Zic3* locus. In addition, no expression of *Zic3* can be detected by RT-PCR in affected *Bn* males. Human mutations in *ZIC3* are associated with left-right axis malformations. Similar anomalies were found in *Bn* males and females, in both the abdominal and thoracic cavities. The presence of anal and spinal abnormalities in some of the *ZIC3* mutant human patients and the deletion of *Zic3* in *Bn* mice support a key role for this gene in neural tube development and closure.

Program Nr: 287 from the 2000 ASHG Annual Meeting

Genotype-phenotype correlation in malformations caused by mutations in the p63 gene. *P. Ianakiev[1], M.W. Kilpatrick[1], I. Toudjarska[1], D. Basel[2], P. Beighton[2], P. Tsipouras[1].* 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Human Genetics, University of Cape Town, Observatory, South Africa.

Gene targeting studies have demonstrated that p63, a homologue of the cell-cycle regulator TP53, plays a critical role the formation and differentiation of the apical epidermal ridge (AER), a stratified epithelium essential for limb development. Mice lacking p63 have striking developmental defects including limb truncations, abnormal skin and absence of hair follicles, teeth and mammary glands. Split Hand-Split Foot (SHFM)is a limb malformation involving the central rays of the autopod presenting with median clefts of the hands and feet, abence of fingers and toes and syndactyly. There is phenotypic overlap with the EEC (ectrodactyly, ectodermal dysplasia, facial cleft) syndrome, in which distal limb malformations sometimes occur as part of the phenotypic spectrum. We have identified two missense mutations, 724A>G predicting an amino-acid substitution K194E and 982T>C predicting an amino-acid substitution R280C, in exons 5 and 7 respectively of the p63 gene in two families with SHFM and two mutations in families with EEC syndrome; 279R>H and 304R>Q. All four mutations fall within the DNA binding domain of the p63 molecule. The possible consequences of the mutations were assessed by building a model of the DNA binding domain of the p63 protein. The two amino-acids mutated in the SHFM families appear primarily to be involved in maintaining the overall structure of the domain, whereas the p63 mutations responsible for EEC syndrome reside in amino acid residues that directly interact with the DNA. This raises the possibility that the phenotypic distinction between SHFM and EEC syndrome arise as a consequence of this difference. The Adams-Oliver syndrome (AOS),in which variable distal limb reduction abnormalities occur in conjunction with defects of the calvarium and scalp, also overlaps phenotypically with SHFM and EEC. In addition to expanding our analysis of the p63 gene in SHFM and EEC families, we are investigating its putative role in AOS. In this way we hope to further understand the relationship between different mutations in the p63 gene and the phenotypes they produce.

Program Nr: 288 from the 2000 ASHG Annual Meeting

TGFb **mediates impaired alveologenesis in fibrillin-1 deficient mice.** *E.R. Neptune[1], P.A. Frischmeyer[1], D.E. Arking[1], T.E. Bunton[1], L. Myers[1], F. Ramirez[2], H.C. Dietz[1,3]*. 1) Institute of Genetic Medicine, Johns Hopkins SOM, Baltimore, MD; 2) Mt. Sinai SOM, New York, NY; 3) HHMI.

Marfan syndrome (MFS) is a heritable disorder of connective tissue caused by mutations in fibrillin-1, a constituent of extracellular microfibrils. Although the major manifestations are in the cardiovascular and skeletal systems, ~11-15% of pts manifest lung disease characterized by emphysematous changes which predispose to spontaneous pneumothorax. Current pathogenetic models invoke a destructive process that is initiated by physiologic stress acting upon a tissue that lacks structural integrity. We have now had the opportunity to test this hypothesis in fibrillin-1 deficient mice that are homozygous for targeted Fbn1 alleles and recapitulate the vascular phenotype of MFS. A striking deficiency in distal septation of alveoli was evident at birth and maintained until the time of vascular death (~10 PD). An intermediate phenotype was observed in the heterozygous animals. Elastin deposition was preserved and ultrastructural analysis revealed normal distribution of elastin at the tip of primordial septae. In that excessive TGFb1 signaling was previously associated with alteration of distal branching morphogenesis and fibrillin-1 contains domains with homology to latency-inducing TGFb binding proteins, we hypothesized a role for this family of cytokines in the lung phenotype of MFS. An antibody specific for active TGFb1 was used to demonstrate a dramatic increase in immunoreactivity within the lungs of fibrillin-1 deficient mice. We utilized a novel transgenic reporter allele, comprised of tandem TGFbeta-responsive promoter elements upstream of the gene encoding green fluorescent protein, to document a 4-fold and 25-fold increase in TGFb signaling in vivo in heterozygous and homozygous Fbn1-targeted mice, respectively. Intraperitoneal injection of TGFb neutralizing antibody at birth rescued lung branching in the heterozygote animals. These data document a role for fibrillin-1 in modulating local active concentrations of TGFb and demonstrate that perturbation of this regulation underlies primary failure of lung branching morphogenesis and perhaps other manifestations of MFS.

Program Nr: 289 from the 2000 ASHG Annual Meeting

Mice doubly mutant in the Fanconi Anemia group A and C genes are viable, but display more severe sensitivity to DNA crosslinkers than either mutant alone. *M. Noll[1], K. Battaile[1], R. Bateman[1], C. Reifsteck[1], S.B. Olson[1], Y.M.N. Akkari[1], K. Rathbun[2], G. Bagby[2], A. D'Andrea[3], M. Grompe[1].* 1) Dept Molec/Medical Gen, L103, Oregon Health Sci Univ, Portland, OR; 2) VA Hospital, Portland, OR; 3) Harvard Medical Institute, Boston, MA.

Fanconi's Anemia (FA) is an autosomal recessive disorder characterized by birth defects, increased incidence of malignancy and progressive bone marrow failure. At least 8 complementation groups exist (FANCA-H) and 4/8 FA genes have been cloned. Here, we report the creation of a FANCA knock-out mouse, with a deletion of exon 37 resulting in a frame-shift and absent protein. FANCA is the most common defect in human FA, representing approximately 65% of all patients. Similar to FANCC mutants FANCA mutant mice were viable and had no gross developmental defects, but showed mild germ cell loss and hypersensitivity to DNA cross-linking agents. No hematologic abnormalities or cancer were observed. We next generated animals deficient in both FANCA and FANCC. Double mutants were born at the expected frequency and no macroscopic developmental abnormalities of the limbs or other organs were detected. Next, we established primary ear fibroblast cultures from FANCA, -C and A/C double mutants and control littermates. Treatment with MMC and DEB mutant cells revealed increased chromosome breakage in A/C double mutants compared to either mutant alone. This was corroborated by cell cycle analysis of primary cultures of MEFs exposed to 8-methoxypsoralen. Double mutant fibroblasts were at least five-fold more sensitive to the cross-linkers than the single mutants. Furthermore, histological analysis of both testis and ovaries showed a more profound germ cell loss in double mutants. Testicular weight was significantly reduced in the double mutants (101 mg) as compared to either FANCA -/- (158 mg), FANCC -/- mice (177 mg) or controls (214mg). Even at 6 months of age, no hematologic abnormalities or tumors have been observed A/C double mutants. We thus conclude that the FANCA and C proteins are at least partially non-epistatic and that mutation of either gene alone, does not render the FA dependent crosslinker response completely inactive.

Program Nr: 290 from the 2000 ASHG Annual Meeting

***ATM* mutation detection in lymphomas using oligonucleotide microarrays.** *J.G. Hacia[1], N.Y. Fang[1], T.C. Greiner[2], J.O. Armitage[2], W.C. Chan[2], J. Vose[2], D. Weisenburger[2], R.A. Mayer[2], F.S. Collins[1]*. 1) GMMB, NHGRI/NIH, Bethesda, MD; 2) UNMC, Omaha, NE.

Ataxia telangiectasia (A-T) is an autosomal recessive disorder characterized by neurological degeneration, immune deficiency, and cancer predisposition. A-T patients demonstrate a 250-fold excess of lymphomas relative to the general population. To investigate the role *ATM* plays in the development and progression of sporadic lymphomas, we are analyzing over 120 DNA samples from follicular, mantle cell, diffuse large cell, peripheral T-cell, and post-transplant lymphomas for *ATM* mutations. We aim to identify subtypes commonly carrying ATM mutations and correlate this data with disease prognosis and responsiveness to treatment. Given the large size of the 9.4-Kb *ATM* coding region and its complex mutational spectrum, it is a challenge to rapidly and inexpensively scan for all possible sequence changes. We developed oligonucleotide microarray (DNA chip) assays to accomplish this task. *ATM* coding exons from patients and unaffected controls were added to DNA chips consisting of over 250,000 different probes. Mutations are detected through increased or decreased patient DNA hybridization to the arrayed probes relative to controls. Mutated exons identified by the DNA chip assays are sequenced to confirm the nature of the sequence chance.

Over 90 lymphoma DNA samples have been screened for *ATM* mutations. A total of 5/25 mantle cell lymphoma samples showed deleterious mutations. These included three nonsense mutations and an in-frame deletion that removes two conserved amino acids. Two samples had missense mutations in conserved amino acids. No increased incidence of clearly deleterious mutations have been found in the other lymphoma subtypes.

We have successfully demonstrated the application of DNA chips to large-scale *ATM* mutation detection in tumor samples. *ATM* mutations may play a role in the development or progression of mantle cell lymphoma. We will evaluate germ-line mutational status to determine if carriers have an increased risk of developing certain lymphoma subtypes.

Program Nr: 291 from the 2000 ASHG Annual Meeting

Duplication history of a subtelomeric block containing an expressed olfactory receptor gene. *H.C. Mefford[1], L. Linardopoulou[2], D. Coil[2], G. van den Engh[2], B.J. Trask[1,2].* 1) Genetics, University of Washington, Seattle, WA; 2) Molecular Biotechnology, University of Washington, Seattle, WA.

We have used sequence analysis to deduce the evolutionary history of a block of sequence duplicated on multiple chromosomes. Some duplication events are recent enough that the block is polymorphic in number and location. Individuals may have 7-11 copies in their genome. Because the block contains at least one expressed olfactory receptor gene, OR-A, its polymorphism may have phenotypic consequences. The complex subtelomeric structure suggests that multiple exchanges and/or duplications may have occurred among non-homologous chromosomes. To gauge the frequency of such exchanges and to determine the potential function of the many copies of OR-A, we compared 2kb of sequence, 1kb of which is the OR-A coding exon, from 154 chromosomes from 19 individuals from 6 populations. Of 19 polymorphic sites in the exon, 10 are nonsynonymous, and 1 causes a premature stop. The 20 ORF-encoding haplotypes may be translated into13 slightly different proteins. Individuals may have from 3-7 protein haplotypes in varying copy number. Average divergence from chimp is 0.83% in noncoding DNA and 0.72% at OR-A. Nucleotide diversity in noncoding DNA is greatest on chromosomes 16 (0.38%) and 19 (0.33%), suggesting that copies at these locations may be the oldest (2.8 My and 1.9 My, respectively). Chromosome 7 alleles may also be old (1.8 My) as they show great diversity in OR-A, but surprisingly little noncoding diversity. Chromosome 3 is least diverse, with 37 of 40 alleles identical across the entire 2kb. This result is surprising since FISH shows that the block is fixed on chromosome 3 in all populations analyzed. Of note, the block is found on chromosomes 7 and 16 almost exclusively in African Pygmy populations. Our analyses provide evidence for exchange, albeit infrequent, between non-homologous chromosomes. We detect one ectopic gene conversion event between chromosome 3 and 15. Our results also suggest that copies of the block were transferred to each of chromosomes 5, 8 and 11 from at least two separate sources by non-homologous exchange.

Program Nr: 292 from the 2000 ASHG Annual Meeting

Recombination hotspots for NF1 microdeletions. *K. Stephens[1], M.O. Dorschner[1], C.L. Friedman[2], B.J. Trask[2], V.P. Sybert[3].* 1) Dept of Medicine; 2) Dept of Molecular Biotechnology; 3) Dept of Dermatology, University of Washington, Seattle, WA.

Patients with 1.5 Mb neurofibromatosis 1 (NF1) microdeletions are remarkable for an early age at onset and large numbers of dermal neurofibromas. The clustering of breakpoints in ~100 kb flanking paralogous DNA segments (NF1REPs) suggested that these deletions arose by homologous recombination. Breakpoint mapping of 11 patients revealed two recombination hotspots. The hotspots are 20 kb apart and each is 2-3 kb in length. At one of these hotspots, the majority of breakpoints fall at or within two GA-rich regions. This region has an upstream CpG island, several LINE elements and retroviral LTRs, but no transposons or open reading frames. Although the breakpoints could have occurred anywhere in the NF1REPs, which share >97% sequence identity, only specific sites were recombination-prone. We hypothesize that the GA-rich regions facilitate recombination via localized effects on DNA secondary structure. SNP analysis surrounding the breakpoints revealed chimerism indicating that the recombinant NF1REP consisted of piecemeal fragments of the two flanking parental NF1REPs. This supports a model of homologous recombination between NF1REPs followed by double strand break repair. The human genome contains at least 9 paralogs belonging to the NF1REP family of sequences. The two REPs that mediate NF1 microdeletions are in direct orientation and share ~50 kb of near identical sequence along with an additional ~30 kb that are shared with other family members but not with each other. FISH and BLAST analyses mapped a third NF1REP at the NF1 gene region (17q11.2) and 5 additional paralogs on chromosome 17 with two at band q12, two at q21 and one at q24. A ninth paralog is at chromosome 19p13. None of these are identical in structure but are mosaics of subrepeats resulting from regional-specific duplication events during evolution. NF1REP-mediated rearrangements may provide a model system for the identification of sequence motifs that predispose to mammalian homologous recombination.

Program Nr: 293 from the 2000 ASHG Annual Meeting

Structure and primate evolution of the SMS-REP repeat gene clusters. *S.S. Park[1], J.R. Lupski[1,2]*. 1) Dept. of Molecular Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept. of Pediatrics and Texas Children's Hospital, Baylor College of Medicine, One Baylor Plaza, Houston, TX.

The Smith-Magenis syndrome (SMS) is a contiguous-gene-deletion syndrome associated with del(17)(p11.2p11.2). The majority (>90%) of patients are deleted for the identical genomic region believed to encompass ~5 Mb. Physical mapping studies demonstrate the presence of large (>200kb), region specific, low-copy-repeat gene clusters (SMS-REPs) containing at least 4 genes (*CLP*, *TRE*, *SRP*, *KER*) and flanking the common deletion interval of SMS. Homologous recombination using SMS-REPs as substrates is responsible for SMS deletion, and the reciprocal recombination may be responsible for duplication, dup(17)(p11.2p11.2), of this same region. As an initial step in the complete sequence analysis of the three SMS-REPs, the flanking proximal (SMS-REPP) and distal (SMS-REPD) as well as the middle (SMS-REPM) repeats, we performed physical mapping of these regions. In addition to physical mapping, we studied the evolution of SMS-REPs during primate speciation. Genomic clones were identified using SMS-REP probes (*CLP*, *TRE*, *SRP*) to screen a human BAC library (RPCI-11). The isolated BAC clones were then mapped to a specific SMS-REP using several structural differences (*cis*-morphisms) between SMS-REPs on the same chromosome (*Hin*dIII with *CLP*; *Hin*dIII, *Xba*I, and *Pst*I with *TRE*; *Pvu*II, *Rsa*I, and *Taq*I with *SRP*). In this manner, we isolated 18 clones in SMS-REPP, 24 clones in SMS-REPD, and 11 clones in SMS-REPM, and constructed a contig for each SMS-REP. By direct DNA sequencing of these BAC contigs in collaboration with the Whitehead Genome Sequencing Center, we will determine the structure of SMS-REPs. To analyze the evolution of SMS-REPs, we performed Southern blotting with a CLP probe using the DNAs of primates (6 species of Apes, 4 of Old World Monkey, and 2 of New World Monkey). Proximal SMS-REP specific *cis*-morphism was identified in Gorilla, Chimpanzee and Pygmy chimpanzee, while distal SMS-REP specific *cis*-morphism was not identified in Gorilla, suggesting that the distal SMS-REP represents a genomic duplication of the progenitor proximal SMS-REP that occurred during primate speciation.

Program Nr: 294 from the 2000 ASHG Annual Meeting

A high-density whole genome association study of calcium entry into lymphoblasts. *N.J. Schork[1], J. Gardner[2], A. Aviv[2]*. 1) Department of Epidemiology and Biostatistics, Case Western Reserve Univ, Cleveland, OH; 2) The Hypertension Research Center, New Jersey College of Medicine & Dentistry, Newark, NJ.

Whole genome association studies that make use of a high-density map of markers have been put forward recently as realistic strategies for the identification of chromosomal loci and regions which harbor genes that influence complex human traits and diseases. Unfortunately, there is little practical experience to support this claim. We describe a study investigating the relationship between 8,236 microsatellite markers dispersed throughout the genome and the rate of calcium entry into immortalized lymphoblasts from 5 CEPH families. Although our study resulted in the identification of many candidate loci that may influence calcium entry, in this presentation we focus on our attempts to deal with many issues that will plague whole genome association studies, such as the choice of analysis models and test statistics and the evaluation statistical significance. Our study is the first high-marker-density whole-genome association study for a human quantitative trait, and as such demonstrates issues worth further consideration as well as documenting the utility of such a study.