MOLECULAR BIOLOGY OF HUMAN GENETIC DISEASE

Organizers: Maimon Cohen, Beverly Emanuel, David Ledbetter and Arthur Beaudet

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Plenary Sessions *Page*

Poster Sessions

Diseases Manifesting Novel Inheritance Patterns

D 001 COMPLEX MOLECULAR MECHANISMS AND MULTIPLE GENES ARE INVOLVED IN IMPRINTING IN PRADER-WILLI AND ANGELMAN SYNDROMES.

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Our studies aim to identify the mechanisms and genes involved in imprinting in mammals and human disease. We have previously shown that Prader-Willi (PWS) and Angelman (AS) syndromes are excellent models for genomic imprinting (reviewed in ref. 1), based on differential parental origins of 15q11-q13 deletions or of uniparental disomy (UPD) for chromosome 15 (reviewed in ref. 2), and have identified the first DNA methylation imprint in an endogenous gene (*ZNF127*) in humans (3). The following six types of new observations suggest that imprinting is more complex and involves more genes than previously thought. First, we have identified three functionally imprinted genes that are potentially involved in PWS. The genes encoding a novel putative polypeptide as well as the previously identified SmN are not expressed in PWS patients with only a maternal allele(s) present, but are expressed in normal or AS individuals. Similarly, the paternal *Znf127* allele but not the maternal allele is expressed in brains from the F_1 offspring of a 129/Rl X *M. spretus* cross in mice. Furthermore, a 2.7-kb *Znf127* mRNA is expressed in undifferentiated embryonic stem (ES) cells and in primary embryonic fibroblasts (PEF's) derived from normal or androgenetic (paternal chromosomes only) embryos, but not in PEF's derived from parthenogenetic (maternal chromosomes only) embryos. We are currently analyzing the phenotypic effect of mutations in *Znf127* in the mouse and whether expression of the *ZNF127* gene is imprinted in humans. Second, the former two genes have a novel mechanism of regulation, rare in higher eukaryotic organisms. Third, the expression of each of these human genes correlates with DNA methylation imprints. Fourth, we have identified a unique family with affected AS sibs who have no deletion or UPD of 15q11-q13, but who have methylation imprints typical of AS patients (ie., paternal only despite biparental inheritance) at three loci separated by over 1 Mb; a probable and intriguing hypothesis is a mutation in the imprinting process (4). Fifth, we have shown that chromosome deletions and rearrangements can alter DNA methylation imprints for distant loci in *cis*, presumably by a position effect on *de novo* or maintenance DNA methylation (4). Lastly, our studies have shown that imprinted genes are embedded within large differentially replicated chromosomal domains, in which the paternal allele is always early replicating (5). Our studies indicate that the number of imprinted genes is likely to be significantly larger than previously thought. Furthermore, the mechanism of imprinting appears to involve a complex interplay of events involving higher order chromatin structure and function of a large imprinted chromosomal region, with gene-specific events, including DNA methylation, occurring at multiple genes within each imprinted domain.

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D 002 OVERVIEW AND UPDATE ON FMR1 AND FRAGILE X SYNDROME, Stephen T. Warren, Howard Hughes Medical Institute and Departments of Biochemistry and Pediatrics, Emory University School of Medicine, Atlanta, GA 30322.

Fragile X syndrome is the most frequent inherited form of mental retardation and is inherited as an X-linked dominant disorder with reduced penetrance. This disorder is the result of a unstable CGG-repeat within the 5' UTR of the gene FMR1. In normal individuals, this repeat is highly polymorphic in length, with the most frequent allele being 30 repeats, and is inherited in a stable fashion. Among affected males and females, this repeat is massively expanded, usually beyond 700 repeats, while unaffected male carriers and most female carriers have repeats of intermediate length, varying from approximately 52 to 200 repeats. Within a fragile X pedigree the repeat is exquisitely unstable when transmitted resulting in a mutation rate approaching one. The repeat tends to increase upon transmission and only undergoes the full expansion into the penetrant range when maternally inherited with a risk in direct proportion to the maternal repeat length. Linkage disequilibrium studies suggest the existence of specific normal haplotypes which are predisposed toward the fragile X mutation. Sequence analysis of the repeats of various haplotypes indicate that the CGG-repeat is normally cryptic, interspersed with AGG triplets and that lengthy pure CGG-repeats may be the predisposing factor. Concomitant with repeat expansion beyond 230 repeats, the FMR-1 gene and promoter become heavily methylated resulting in transcriptional silencing. Absence of the FMR-1 protein (FMRP) is therefore believed to result in the fragile X phenotype. Recent studies on FMRP indicate this to be an RNA-binding protein containing conserved KH-domains and RGG boxes. FMRP is selective in RNA interaction, binding to approximately 4% of human brain message. This suggests that other genes, whose products may be varied by the absence of FMRP, could play a consequential role in the pleiotropic phenotype of fragile X syndrome.

Cancer Genetics (Joint)

D 003 THE MOLECULAR BASIS OF THE t(2;13) TRANSLOCATION IN THE PEDIATRIC SOLID TUMOR ALVEOLAR RHABDOMYOSARCOMA,

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Alveolar rhabdomyosarcoma is an aggressive soft tissue tumor which occurs in the pediatric population. Histologic diagnosis is often complicated by the paucity of features of striated muscle differentiation and its similarity to a large group of pediatric solid tumors which can present as collections of poorly differentiated small round cells. Cytogenetic investigations of this tumor have identified a characteristic translocation involving chromosomes 2 and 13, t(2;13)(q35;q14). Identification of the genetic loci disrupted by the t(2;13) translocation will permit investigation of an important step in the pathogenesis of rhabdomyosarcoma and exploration of the utility of this molecular marker/target in clinical management. We developed a physical mapping strategy to localize the tumor translocation breakpoint sites on genetic maps of chromosome 2 and 13. This strategy facilitated identification of chromosome 2 and 13 probes which closely flank the breakpoint and delineation of physical intervals which contain the breakpoint. Using these reagents, we have identified the genes involved in the translocation. We have determined that PAX3 (a developmentally regulated transcription factor previously found to be mutated in Waardenburg syndrome) is the chromosome 2 locus rearranged by the t(2;13) translocation. The involved gene on chromosome 13 (FKHR) has been subsequently cloned and identified as a new member of the *fork head* domain family of transcription factors characterized by a conserved DNA binding motif. Northern blot analysis has shown that the juxtaposition of the PAX3 and FKHR genes results in a chimeric transcript consisting of the 5' PAX3 and 3' FKHR regions. PCR analysis has demonstrated that a uniform 5' PAX3-3' FKHR chimeric transcript is expressed in 8 of 8 alveolar rhabdomyosarcoma cell lines. This chimeric transcript encodes a fusion protein consisting of the intact PAX3 DNA binding domains (paired box and homeodomain), the C-terminal half of the *fork head* DNA binding domain, and C-terminal FKHR regions. This fusion protein has been detected in alveolar rhabdomyosarcoma cells by immunoprecipitation experiments with PAX3 and FKHR-specific antisera. These findings demonstrate that the t(2;13) translocation of alveolar rhabdomyosarcoma fuses functional domains from two transcription factors to generate a potentially tumorigenic hybrid transcription factor.

D 004 MOLECULAR GENETICS OF NEUROFIBROMATOSIS TYPE 1, Francis S. Collins[1], David H. Gutmann[2], Anna L. Mitchell[2], Steve Doran[2], Lone B. Andersen[2], Paula E. Gregory[2], Amit Hajara[2], Manju Swaroop[2], Jeffrey Cole[2], [1] National Center for Human Genome Research, National Institutes of Health, [2] University of Michigan, Ann Arbor.

Type 1 neurofibromatosis (NF1) is a common autosomal dominant disorder characterized by café-au-lait spots, neurofibromas over the skin surface, Lisch nodules of the iris, and a variety of other variable features including an increased risk of malignancy. The gene for NF1 was identified by a positional cloning strategy in 1990, allowing for the first time the opportunity to understand the disease on the molecular level. We have been investigating the genetic basis of the disease by searching for germline abnormalities in affected individuals. The large size of the gene, the fact that most mutations are subtle changes, and the fact that virtually every family appears to have a different mutation still prevent DNA diagnosis from being widely applicable. Mutations which have been identified primarily appear to be knockouts, which is consistent with a tumor suppressor gene mechanism. We have recently developed fluorescence in situ hybridization techniques to detect large deletions in the gene. In one large pedigree with apparent non-penetrance, we were able to show utilizing FISH that there are actually two independent de novo *NF1* mutations. In another NF1 patient a paracentric inversion of 17q was found to break within the *NF1* locus, providing an unusual mechanism for disease. We have also investigated somatic alterations in the *NF1* gene in tumors. The results are consistent with a tumor suppressor mechanism, wherein the malignant tumors arising in patients with NF1 appear to have sustained a second hit in the *NF1* locus. Sporadic tumors, especially melanomas and neuroblastomas, also have been found to harbor *NF1* somatic mutations, suggesting that this gene plays a larger role in malignancy. The protein product of the *NF1* gene is 2818 amino acids and encodes a protein (called neurofibromin) with homology to the GTPase activating protein (GAP) family. This protein interacts with p21-ras to catalyzesits conversion from the active GTP-bound state to the inactive GDP-bound state. We have raised antisera against neurofibromin, and recent immunofluorescence data indicates that the protein, is localized in the cytoplasm and appears to be associated with microtubules. Expression of various segments of neurofibromin in a baculovirus system for microtubule association maps to the GAP homology region. This unexpected connection between ras-mediated signal transduction and the cytoskeleton suggests that neurofibromin may play multiple roles in the regulation of cell division.

Human Disease "Knockouts": Animal Models

D 005 GENE TARGETING FOR CD18, ICAM-1, AND P-SELECTIN YIELDS MICE WITH IMPAIRED INFLAMMATORY AND IMMUNE RESPONSES, Arthur L. Beaudet[1,4], Daniel C. Bullard[1], Eric T. Sandberg[2], James E. Sligh[1], Susan S. Rich[3], Allan Bradley[1,4], Claire M. Doerschuk[5], and C. Wayne Smith[2], [1]Institute for Molecular Genetics, and Departments of [2]Pediatrics and [3]Microbiology and Immunology, Baylor College of Medicine and [4]Howard Hughes Medical Institute, Houston, TX 77030, and [5]Department of Pediatrics, Indiana University, Indianapolis, IN 46202

Gene targeting was used in mouse embryonic stem cells to produce mutations in CD18, ICAM-1, and P-selectin. The CD18 mutation is an insertion resulting in a hypomorphic allele with expression from a cryptic promotor in the plasmid construct. Homozygous mutant mice are viable and fertile, demonstrate a mild granulocytosis, and have 2 or 16 percent of normal CD18 expression on granulocytes in the resting or activated state, respectively. The ICAM-1 mutation is a null allele resulting in loss of detectable surface expression on lymphocytes and in absence of ICAM-1 on immunohistochemistry of tissues such as lung. ICAM-1 deficient animals are also viable and fertile and express a mild resting granulocytosis. P-selectin mutant mice should represent a null allele based on the mutation introduced, are viable and fertile, and are currently being characterized further. For CD18 and ICAM-1 mutant mice, T cell enriched splenocytes were isolated and showed marked reduction of homotypic aggregation when stimulated by ionomycin and phorbol myristate acetate. In mixed lymphocyte cultures, ICAM-1 mutant cells failed to stimulate proliferation of allogeneic responders, although the ICAM-1 mutant T cells were capable of responding normally to allogeneic stimulation. Contact hypersensitivity was assessed in CD18 and ICAM-1 mutant mice by the sensitization of the skin to dinitrofluorobenzene. Ear swelling was measured 24 hours after challenge and was significantly decreased in both mutant groups compared to wild type animals (wild type = 16 ± 7; ICAM-1 = 5 ± 5; CD18 = 5 ± 4 mm X 10^{-2}). Ear biopsy demonstrated extensive leukocytic infiltration and edema in wild type animals, but this was absent in both mutant groups. These mice will be useful for the analysis of the role of leukocyte and endothelial cell adhesion molecules in inflammatory disease processes including atherosclerosis, myelin basic protein-induced encephalomyelitis, and collagen induced arthritis.

D 006 THE EFFECTS OF GENETICALLY ALTERED SUPEROXIDE DISMUTASE ACTIVITY. Charles J. Epstein[1], Ting-Ting Huang[1], Pak H. Chan[1], Jean L. Cadet[2], and Elaine J. Carlson[1]. [1]University of California, San Francisco and [2]National Institute on Drug Abuse, Baltimore.

Oxygen free radicals have been implicated in many pathological processes affecting the brain and other organs and tissues, but it has not always been possible to examine their roles directly. One approach to such an examination is to perturb one or more of the components of the metabolic pathway that detoxifies free radicals. Transgenic animal technology and homologous recombination techniques have made it possible to constitutively alter the level of CuZn-superoxide dismutase (CuZnSOD), the first enzyme in the detoxification of superoxide anions, and to examine the effects of such an alteration on the response to agents, both physical and chemical, that are thought to produce deleterious effects through the mediation of oxygen radicals. These alterations in CuZnSOD activity have taken on additional interest with the recent discovery of mutant forms of CuZnSOD in familial amyotrophic lateral sclerosis.

The activity of CuZn-superoxide dismutase in the brain and other tissues of mice has been increased between 1.5- and 5-fold by the insertion of one or more human CuZn-SOD transgenes into the mouse genome. These transgenic mice exhibit several differences from control mice when exposed to known or presumed forms of oxidative stress. They are more resistant to the damaging effects, as determined by infarct size, volume of edema, and degree of vascular permeability, produced by cold injury, contusive injury, and both permanent and transient focal cerebral ischemia. Furthermore, following focal ischemia, the transgenic animals display a more extensive and greatly prolonged production of hsp70 mRNA in the ipsilateral cortex and hippocampus. Transgenic animals are also more resistant to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and of methamphetamine, as manifested by depletion in the levels of dopamine and its metabolites.

Taken together, our findings indicate that elevated CuZn-superoxide dismutase activity in the brain is protective against a variety of forms of acute oxidative stress. This protection may result from interference with 1) reactions in which superoxide radicals and hydrogen peroxide give rise, in the presence of iron, to highly reactive hydroxyl radicals, and 2) the combination of superoxide with nitric oxide to form the peroxynitrite, another source of hydroxyl radicals. The long term effects of altered superoxide dismutase activity remain to be determined. Work is currently in progress to assess the effects of reduced CuZnSOD activity, particularly in the central nervous system.

D 007 FUNCTIONAL ANALYSIS OF THE LDL RECEPTOR GENE FAMILY BY GENE DISRUPTION AND GENE TRANSFER IN THE MOUSE, Thomas Willnow[1], Shun Ishibashi[1], Dennis Burns[2], Hideo Otani[1], Robert E. Hammer[3], Michael S. Brown[1], Joseph L. Goldstein[1], and Joachim Herz[1]; [1] Departments of Molecular Genetics and Internal Medicine, [2]Department of Pathology, [3] Howard Hughes Medical Institute and Department of Biochemistry, University of Texas, Southwestern Medical Center, Dallas TX 75235

The LDL receptor gene family is an expanding group of cell surface receptors which at the current time comprises the low density lipoprotein (LDL) receptor, the LDL receptor-related protein (LRP), the Heymann's nephritits antigen gp330 and the VLDL receptor. All these proteins are composed of the same basic structural motifs: complement-type cysteine-rich repeats, epidermal growth factor precursor-homologous domains, a single transmembrane domain and a short cytoplasmic tail containing between one and three 'NPxY' endocytosis signals. LDL receptor-related proteins have been structurally perfectly conserved throughout evolution, from *Caenorhabditis elegans* to man. As constitutively endocytosed cell surface receptors they are involved in diverse biological processes, including the metabolism of lipoproteins and lipoprotein lipase, plasminogen, plasminogen activators and their inhibitors, α_2-macroglobulin, toxins, lactoferrin and vitellogenin. In some cases, receptor-activity can be modulated by a receptor-associated protein (RAP), a small protein that resides in intracellular compartments. We have begun to study the functional consequences of genetic defects of the individual members of this gene family by gene disruption in mice. The genetically altered animals display several phenotypes including early embryonic lethality, elevated plasma cholesterol levels and formation of atherosclerotic lesions. Furthermore, we have used adenovirus-mediated somatic cell gene transfer to transiently reverse the phenotype caused by the disruption of the LDL receptor gene in the adult animal.

D 008 GENE TARGETING IN MICE AS A STRATEGY FOR UNDERSTANDING LIPID METABOLISM AND ATHEROSCLEROSIS, Nobuyo Maeda, Department of Pathology, University of North Carolina, Chapel Hill, NC 27599-7525.

Atherosclerotic cardiovascular disease, one of the major causes of death in Western society, results from complex interactions between multiple genetic and environmental factors. With the aim of dissecting some of the molecular details of atherogenesis, we have generated mice carrying mutations in the genes in lipid metabolism-related genes.

Apolipoprotein A-I (ApoA-I) is the major protein component of high density lipoprotein (HDL) particles. Decreased plasma levels of HDL cholesterol are correlated with an elevated risk of coronary disease in humans. Mice lacking apoA-I have total plasma cholesterol and HDL cholesterol approximately one-third and one-sixth of those in their normal litter mates. However, no signs of spontaneous atherosclerosis in mutants maintained on regular mouse chow have been observed. Feeding the high fat/high cholesterol diet to mutant and normal mice increased their plasma cholesterol levels and they developed small fat depositions, a sign of early atherosclerotic lesions, in their proximal aorta after 20 weeks of feeding. The occurrence or extent of the depositions were, however, **not** related to the apoA-I genotype. The results imply that a simple reduction of apoA-I does not by itself cause atherosclerosis even on a high fat/high cholesterol diet.

In contrast, mice lacking apolipoprotein E (apoE), which have five times normal plasma cholesterol levels, develop foam cell-rich deposits in their proximal aorta by 3 months of age even when fed regular low fat/low cholesterol mouse chow. ApoE is a constituent of all lipoproteins except low density lipoproteins. It functions as a ligand for receptors that clear remnants of chylomicrons and very low density lipoproteins. Accumulation of these remnant particles is, therefore, atherogenic. The atherosclerotic lesions in mice lacking apoE progress with age. By 10 months of age, lesions were widely distributed throughout the arterial tree, and were observed in coronary, carotid and iliac arteries. These older arterial lesions show increased complexity, including fibrous caps, cholesterol clefts, calcified deposits and microthrombi, equivalent to those observed in advanced atherosclerotic lesions in humans.

Mice producing a truncated form of apolipoprotein B (apoB70) but not apoB100, model hypobetalipoproteinemia in humans. Unexpectedly, some of the homozygous mutants die of developmental abnormalities, exencephalus and hydrocephalus, which may be associated with the malabsorption of fat soluble vitamins.

Homologous Recombination/Mouse Models (Joint)

D 009 THE ROLE OF DNA METHYLATION IN MAMMALIAN DEVELOPMENT, Rudolf Jaenisch, En Li, Peter Laird, Laurie Jackson-Grusby, Ruth Jüttermann, and Caroline Beard, Whitehead Institute for Biomedical Research, Cambridge, MA 02142 and Department of Biology, Massachusetts Institute of Technology, Cambridge.

We have generated a mouse strain carrying a mutated DNA methyl transferase gene. This mutant mouse strain was used to investigate whether DNA methylation is involved in the maintenance of gene inactivity of imprinted genes. For this we have measured the transcriptional activity of Igf-2 which is maternally imprinted and of H19 and Igf-R which are paternally imprinted. Contrary to expectation, homozygous mutant embryos did not express the Igf-2 or Igf-R gene but expressed, in addition to the maternal H19 allele, also the paternal H19 allele. This indicates that interference with DNA methylation activates the normally inactive (imprinted) paternal H19 allele. H19 represents, therefore, the first example of a gene whose expression *in vivo* is dependent on DNA methylation. Expression of Igf-2 may not be directly controlled by DNA methylation but might rather be inhibited in cis by an active H19 allele.

D 010 GENETIC ANALYSIS OF TYROSINE KINASES IN MICE, Philippe Soriano, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

To help understand the physiological role of *src* family non-receptor tyrosine kinases, we have generated mutant mice for *src*, *fyn*, and *yes* by gene targeting in ES cells. Despite widespread expression of these kinases in the embryo and the adult, mutant mice display very restricted phenotypes, such as osteopetrosis in *src* deficient mice or T cell receptor signaling and hippocampal defects in *fyn* mutant mice. The absence of more substantial phenotypes suggests functional compensation by each member of the family. In agreement with this hypothesis, crosses between the different mutant strains have failed to produce the expected number of viable double mutant mice, or double mutant *fyn yes* animals survive, but develop glomerulosclerosis.
To understand the importance of regulating kinase activity during development, we have generated mouse mutants for *csk*, a negative regulator of *src* family kinases. This mutation leads to embryonic lethality associated with defects in the neural tube and in the notochord. Cells derived from the mutant embryos exhibit an order of magnitude increase in activity of *src* and *fyn* . To help identify which kinases need to be carefuly regulated during embryogenesis, we have begun crossing the *csk* mutants with the *src*, *fyn*, and *yes* mutants. The results of these experiments will be discussed.

D 011 USE OF THE GENE KNOCKOUT TECHNIQUE IN THE ANALYSES OF LEARNING AND THE WHISKER-BARREL SYSTEM, Susumu Tonegawa[1], Asa Abeliovich[1], Chong Chen[1], Reha S. Erzurumlu[2], Yukiko Goda[4], Sonal Jhaveri[2], John Kim[5], Yuqing Li[1], Richard Paylor[3], Charles F. Stevens[4], Yanyan Wang[4], Jeanne M. Wehner[3], [1]Howard Hughes Medical Institute at Massachusetts Institute of Technology (MIT) Center for Cancer Research, Cambridge, Massachusetts, [2]Dept. of Brain and Cognitive Science at MIT, Cambridge, Massachusetts, [3]Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado, [4]Howard Hughes Medical Institute at Salk Institute, La Jolla, California, [5]Hedco Neurosciences Bldg., University of Southern California, Los Angeles, California.
We have been applying the genetic approach based on the gene targeting technique to the analyses of learning and memory processes. The first mouse mutant produced by this technique, the α CaMKII mutant, exhibited LTP deficiency in the CA1 field of the hippocampus as well as a defect in the spatial learning in the Morris water maze. These results strengthened the notion that the hippocampal LTP is the mechanism responsible for spatial learning. In order to examine the validity of this theme further, we produced a second strain of mutant mice in which the gene encoding the γ isoform of protein kinase C is mutated. The PKC γ mutant mice exhibited at least as much deficiency in the CA1 field LTP as the α CaMKII mutant mice, but their impairment in the spatial learning in the Morris water maze was distinctly lighter than that of the α CaMKII mutant mice. In fact, the impairment in the spatial learning ability of the PKC γ mutant mice could be revealed only by the most sensitive test, namely the platform crossing test after a milder training protocol but not with other tests, such as the quadrant test or random platform test, or with any tests tried after a more intense training program. These results suggest that the CA1 LTP is probably only one of multiple synaptic mechanisms that are responsible for spatial learning. Since the hippocampal LTD is intact in the PKC γ mutants, but is defective in α CaMKII mutants or AP5-treated animals, LTD is a good candidate for one of such mechanisms.
Our second project is to apply the gene targeting technique to the analysis of the activity dependent fine-tuning of neuronal connections. In particular, we have been studying the formation of whisker barrelettes using mice lacking the NMDA receptor (NMDA-R). The mutant mice die shortly (10 to 20 hrs) after birth just around the formation of whisker-related patterns in the brainstem trigerminal complex (BTC). However, we could manage to delay the birth by one to two days. The cytochrome oxidase-stained barrelettes were present in the pups of wild type mice, while they were clearly absent in the mutant pups, despite dense staining in the nuclei. Staining with DiI indicated that the absence of the pattern in the mutant brainstem is not due to delayed growth of trigerminal ganglion axons into the BTC. These results suggest that the formation of barrelettes requires NMDA receptor activation and is activity-dependent.

Human Diseases I (Joint)

D 012 MOLECULAR DEFECTS IN HUMAN EXCISION REPAIR SYNDROMES, Dirk Bootsma, Geert Weeda, Wim Vermeulen, Hanneke van Vuuren, Christine Troelstra, Jan Hoeijmakers, MGC-Department of Cell Biology and Genetics, Erasmus University, Postbox 1738, 3000 DR Rotterdam, The Netherlands.

Nucleotide excision repair (NER) is a universal repair system that eliminates a wide spectrum of DNA lesions. It is of crucial importance for guarding genetic integrity and for preventing the deleterious effects of ubiquitous genotoxic agents such as cancer. In fact, two NER subpathways exist: one dealing with the rapid and efficient removal of lesions that block ongoing transcription (transcription-coupled repair), one accomplishing the more slow and less efficient repair of the bulk DNA, including the non-transcribed strand of active genes (genome overall repair).

The phenotypic consequences of a NER defect in man are apparent from 3 distinct inborn diseases characterized by hypersensitivity of the skin to sun(UV)light and a remarkable clinical and genetic heterogeneity. These are the prototype repair syndrome xeroderma pigmentosum (XP) (7 genetic complementation groups, designated XP-A to -G), Cockayne's syndrome (2 groups: CS-A and CS-B) and PIBIDS, a peculiar photosensitive form of the brittle hair disease trichothiodystrophy (TTD, at least 2 groups of which one equivalent to XP-D).

To investigate the mechanism of NER and to resolve the molecular defect in these NER deficiency diseases we have focussed on the cloning and characterization of human DNA repair genes. One of the genes that we cloned is *ERCC3*. It specifies a chromatin binding helicase. Transfection and microinjection experiments demonstrated that mutations in *ERCC3* are responsible for XP complementation group B, a very rare form of XP that is simultaneously associated with Cockayne's syndrome (CS). In collaboration with the laboratory of J-M. Egly (Strasbourg) the ERCC3 protein was found to be part of a multiprotein complex (TFIIH) required for transcription initiation of most structural genes and for NER. This defines the additional, hitherto unknown vital function of the gene, suspected from parallels with the yeast and Drosophila *ERCC3* homologs. Part of the clinical symptoms of the corresponding disorder that were difficult to interpret on the basis of a DNA repair defect can now be fully explained as the result of a subtle impairment of transcription. It appears, that the NER system recruits for its reaction proteins engaged in replication, recombination and (as in the case of *ERCC3)* in transcription, in addition to specific NER proteins. We will summarize the present status on the biochemical characterization of several of the human NER proteins, the surprising functional relationship between transcription and repair and its implications for the clinical symptoms of the corresponding NER disorders and the use of NER genes to generate corresponding mouse models for human repair deficiencies using gene targeting and embryonal stem cell technology.

Gene Therapy: Somatic Tissue II (Joint)

D 013 SYSTEMIC DELIVERY OF RECOMBINANT PROTEINS BY GENETICALLY ENGINEERED MYOBLASTS, Helen M. Blau and Jyotsna Dhawan, Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, CA 94305-5332.

Studies of pattern formation during development have revealed that myoblasts are particularly advantageous for cell mediated gene transfer, because following injection they randomly fuse with all myofibers in their vicinity and become integrated into a pre-existing structure, the multinucleated myofiber[1,2]. As a result they are in contact with the circulation and sustained by neuronal activity. In a recent clinical trial, biopsies of Duchenne Muscular Dystrophy patients 1 and 6 months after implantation with myoblasts produced the missing gene product, dystrophin detected by PCR[3]. Myoblasts genetically engineered with retroviruses deliver recombinant proteins such as growth hormone to the circulation for at least 3 months[4,5]. Current studies of myoblast mediated gene transfer are directed at showing therapeutic efficacy in an animal model with a heritable defect. This approach appears to have promise both for treating myopathies and inherited and acquired nonmuscle disorders including hemophilia, cancer and heart disease.

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D 014 POSSIBLE ROLES FOR UTROPHIN IN GENE THERAPY OF DUCHENNE MUSCULAR DYSTROPHY, Kay E. Davies, Jon Tinsley, Derek J. Blake, Marcela Pearce, Anna Deconick and Carina Dennis, Institute of Molecular Medicine, Oxford, England.

Duchenne muscular dystrophy (DMD) is a muscle wasting disorder which results from the lack of expression in muscle of a protein, dystrophin, encoded by a very large (>2 megabases) gene at Xp21 on the human X chromosome. Although the localisation of dystrophin at the sarcolemma is well documented, its exact function remains to be defined. Several strategies are being developed for the gene therapy of DMD including myoblast transfer, retroviral infection, adenoviral infection and direct injection of plasmid DNA. Although some progress is being made in all these areas, there are considerable problems to be overcome related to the number of muscles which can be made dystrophin positive, the levels of expression and the duration of expression. The aim of our studies is to develop strategies to compensate for the loss of dystrophin in the muscle cells of patients by upregulating related genes. Our approach is to perform a detailed characterisation of a protein, utrophin, localised on human chromosome 6, which we have shown to be very closely related in sequence and genomic organisation to dystrophin. Utrophin is localised at the neuromuscular junction in normal muscle but has a localisation similar to dystrophin in DMD patients. Utrophin is also expressed at the sarcolemma in human fetal muscle before dystrophin appears. We are characterising the promoter elements of the utrophin gene in order to understand what factors determine its sarcolemmal localisation in certain circumstances and its appearance at the neuromuscular junction in others.

D 015 GENETICALLY MODIFIED MYOBLASTS FOR THE TREATMENT OF ERYTHROPOIETIN-RESPONSIVE ANEMIAS, Eliav Barr[1], Sandeep Tripathy[1], and Jeffrey M. Leiden[1], [1]University of Chicago, Chicago, IL 60637.

Erythropoietin is a 30 kD glycoprotein that regulates erythropoiesis in mammals. In adults, renal tubular cells synthesize and secrete erythropoietin into the systemic circulation under conditions of decreased renal oxygen tension such as occur with anemia. Erythropoietin binds to a receptor on the surface of erythroblasts stimulating both cellular proliferation and differentiation to reticulocytes. Severe anemia due to inappropriate erythropoietin secretion is a common concomitant of both end stage renal disease and symptomatic HIV infection. These erythropoietin-responsive anemias affect more than 150,000 adults in America. Erythropoietin-responsive anemias are currently treated with repeated subcutaneous or intravenous infusions of recombinant human erythropoietin. This therapy is estimated to cost more than $200 million per year. The development of a cell based system that could be used to stably deliver physiological levels of human erythropoietin to the systemic circulation would represent a significant advance in our ability to treat this disease. We have shown previously that genetically modified murine myoblasts can be used to deliver physiologic levels of human growth hormone to the systemic circulation for periods of as long as three months. In the current studies we have (i) isolated and grown primary human myoblasts, (ii) used liposome-mediated gene transfer to transfect these cells with eukaryotic human erythropoietin expression vectors, (iii) shown that these primary human myoblasts secrete high levels (16 U per day per 10^6 cells) of human erythropoietin *in vitro*, (iv) expanded clones of erythropoietin-producing primary human myoblasts to large cell numbers and implanted these cells by intramuscular injection into SCID mouse muscle *in vivo*, and (v) demonstrated that implantation of these genetically modified cells results in significant increases in the hematocrits of these SCID mice. Taken together, these studies suggest that genetically modified myoblasts represent a useful system for the gene therapy of erythropoietin-responsive anemias in humans.

D 016 ß-GLUCURONIDASE GENE TRANSFER IN ANIMAL MODELS OF MUCOPOLYSACCHARIDOSIS TYPE VII John Wolfe[1], Rosanne Taylor[1], Mark Sands[1], Margret Casal[1], Noam Harel[1], Gil-Hong Park[1], Michael Parente[1], John Fyfe[1], Mark Haskins[1], Don Patterson[1], and Evan Snyder[2] [1]University of Pennsylvania School of Veterinary Medicine, Philadelphia PA and [2]Harvard Medical School, Boston MA.

Mucopolysaccharidosis (MPS) type VII (Sly disease) is caused by an inherited deficiency of ß-glucuronidase (GUSB) resulting in failure to degrade glycosaminoglycans (GAGs). The storage of undegraded substrates in lysosomes of many cell types leads to progressive degeneration of multiple organ systems, mental retardation, and early death. Mouse, dog, and cat models of MPS VII have essentially the same pathology and clinical features as human patients. Treatment strategies for lysosomal storage diseases are based on the observation that lysosomal enzymes can be transported from normal cells to mutant cells ("cross-correction") via receptor-mediated uptake or cell-to-cell contact. Thus normal enzyme can potentially be delivered to patients by transplantation of heterologous normal cells, infusion of purified enzyme, or gene transfer to autologous somatic cells. Bone marrow transplantation can produce substantial clinical improvement in the visceral and skeletal disease in MPS VII mice and dogs, especially when performed at a young age, but is not effective in delivering therapeutic levels of GUSB to the brain. Intravenous injection of GUSB results in widespread delivery to the body, including the brain if treatment is begun at birth, but lysosomal storage recurs if treatment is stopped. Retroviral vector-mediated gene transfer in vitro corrects the GUSB deficiency of MPS VII cells, restoring substrate degradation to normal, and the transferred enzyme is exported and cross-corrects untreated target cells. In vivo, retroviral vector-mediated gene transfer to MPS VII hematopoietic stem cells or fibroblasts has shown that long-term expression of only low levels of normal GUSB can produce clinically significant improvements in pathology in liver and spleen, but therapeutic levels of GUSB do not reach the brain. A herpesvirus vector transferred GUSB directly into non-mitotic neurons and long-term expression was achieved using the promoter of the HSV latency associated transcript (LAT) gene, but only a low number of cells were corrected. To attempt to transfer therapeutic amounts of GUSB into the brain of MPS VII animals we are currently using retroviral vectors to transduce autologous cells ex vivo and transplant them by stereotaxic injection into the brain to circumvent the blood-brain barrier. To increase the amount of cross-corrective enzyme delivered, the cells were corrected with a double-copy vector that releases 5-fold higher than normal levels of GUSB, acting as an "enzyme pump." Engrafted cells survive in the brain for more than 6 months, but GUSB expression declines over time, although small numbers of positive cells can be detected long-term. We are also using a neuronal progenitor cell line (C17.2) which may be useful as a general delivery vehicle to transfer foreign genes into the brain. These cells differentiate into neurons, astrocytes, and oligodendrocytes, can be transplanted across MHC barriers, and appear to be non-tumorigenic. Transplantation into the brains of newborns produced widespread engraftment and cytoarchitecturally appropriate differentiation, resulting in GUSB expression for at least 8 months.

Human Diseases II (Joint)

D 017 MOLECULAR GENETICS OF CHARCOT-MARIE-TOOTH DISEASE, Benjamin B. Roa[1], Nacer Abbas[1], Carol A. Wise[1], Kent Anderson[1], Pragna I. Patel[2], and James R. Lupski[1,3,4], Institute for Human Genetics[1], Department of Neurology[2], Human Genome Center[3], and Department of Pediatrics[4], Baylor College of Medicine, Houston, Texas 77030.

Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy. CMT type 1 (CMT1) is characterized by distal muscle atrophy, decreased motor nerve conduction velocity (NCV) and peripheral nerve demyelination. The predominant subtype, autosomal dominant CMT1A, exhibits linkage of the disease locus to DNA markers on proximal 17p. The majority of CMT1A cases are associated with a submicroscopic DNA duplication on 17p11.2-p12. The 3.0-Mb CMT1A duplication consists of a tandem duplication of a 1.5-Mb region. Evidence of a CMT1 phenotype in patients with cytogenetically visible dup(17p) supports a gene dosage mechanism by which the CMT1A duplication leads to the disease. Flanking the 1.5-Mb monomer unit are large (>17kb) homologous sequences (CMT1A-REP) that appear to provide an intrinsic mechanism for recombination leading to the CMT1A duplication of the same size arising in *de novo* CMT1A patients and inherited in CMT1A families of different ethnic origins. In addition, CMT1A-REP appears to be involved in generating the apparent reciprocal deletion of 1.5 Mb associated with hereditary neuropathy with liability to pressure palsies (HNPP), which is clinically distinct from CMT1A. The *PMP22* gene encoding a 22-kDa peripheral nerve myelin protein has been mapped within the CMT1A duplication region and shown to be highly expressed in peripheral nerve. The role of *PMP22* in the demyelinating disease process of CMT1A has been confirmed by the identification of several *PMP22* point mutations in CMT1A patients. These mutations predict single amino acid substitutions in *PMP22*, and include autosomal dominant alleles, as well as an apparent recessive *PMP22* allele contributing to CMT1A. The collective data indicates that Charcot-Marie-Tooth disease type 1A can be caused by two alternative molecular mechanisms: point mutation in the *PMP22* gene, and increased gene dosage due to DNA duplication of the region containing *PMP22*.

Gene Therapy: Somatic Tissue III (Joint)

D 018 DIRECT GENE TRANSFER AND NONVIRAL VECTORS FOR HUMAN CANCER AND AIDS, Gary J. Nabel[1], Bernard A. Fox, Ph.D.[1], Gregory E. Plautz[1], Bei-Yue Wu[1], X. Gao[2], L. Huang[2], Alfred E. Chang[1], Elizabeth G. Nabel[1], [1]University of Michigan, Ann Arbor, MI; [2]University of Pittsburgh, Pittsburgh, PA.

The expression of recombinant genes in living organisms provides potential alternative approaches to the treatment of human diseases. Such approaches can be used, for example, to confer protection against HIV infection by stimulation of the immune system or by preventing infection of otherwise susceptible cells. We have prepared retroviral vectors containing a Rev gene encoding a dominant negative inhibitor. Expression of this gene product confers significant resistance to HIV infection without alteration of normal T cell function. This genetic intervention may provide therapeutic benefits in HIV infection, and a human clinical protocol has recently received NIH regulatory approval to address this question. We have also described a method to stimulate immune responses by expressing a foreign MHC gene in malignant tumors in vivo. By direct gene transfer, a murine class I H-2K^s gene was introduced in vivo into subcutaneous tumors from the CT26 mouse colon adenocarcinoma (H-2^d). Expression of this foreign MHC gene within tumors induced a cytotoxic T cell response to H-2K^s and, more importantly, to other antigens present on unmodified tumor cells. This immune response attenuated tumor growth or caused complete tumor regression in several animals. The results of a clinical trial which utilizes direct gene transfer of a foreign histocompatibility gene for human melanoma will be summarized.

A 208 **INVOLVEMENT OF GP130/IL-6 TRANSDUCING PROTEIN IN IL-11 RECEPTOR.**

Hugues GASCAN, Maryvonne FOURCIN, J.Jacques LEBRUN, John WIDJENES, Annick POUPLARD, Sylvie Chevalier. INSERM U298, ANGERS, INSERM U344, PARIS, Innotherapie,BESANCON, FRANCE.

Recent studies have defined a new emerging family of cytokines sharing biological properties, and which is composed by Interleukin 6 (IL-6), Leukemia Inhibitory Factor (LIF), Oncostatin M (OSM) and Ciliary Neurotrophic Factor (CNTF). Some redundancies of their biological effects is explained by the presence in their multimeric receptor complexes of a common gp130 transducing protein. We studied the possibility for the Interleukin 11 , and which displays many properties of IL-6, to belong to this family. We observed that IL-11 triggered the TF1 erythroleukemic cell line proliferation in a dose dependent manner. The proliferative response to IL-11 was specifically blocked by monoclonal antibodies raised against the gp130 signal transducer. In addition, immunoblotting studies with antiphosphotyrosine antibodies showed that both IL-6 and IL-11 induced tyrosine phosphorylation of gp130. Antibodies against IL-6 receptor/gp80 failed to block the biological activities of IL-11, indicating that the cytokine may use a different receptor binding protein(s) to elicit its effects. Similarly we didn't observe any displacement between IL-11 and IL-6, in competition experiments using radiolabelled IL-6. These results indicate that in addition to gp130 an other binding component is required to mediate the Il-11 activities. These results showed that IL-11, along with IL-6, LIF, OSM, CNTF, belongs to this newly emerging family of cytokines.

A 209 THE DIRECT SYNERGISTIC EFFECTS OF LEUKEMIA INHIBITORY FACTOR ON HEMATOPOEITIC PROGENITOR CELL GROWTH. J.G. Gooya, F.W. Ruscetti and J.R. Keller., BCDP-PRI/Dyncorp, LLB-BRMP, NCI-FCRDC, Frederick, MD 21702.

Leukemia inhibitory factor (LIF) is a multifunctional cytokine that is produced by a variety of cells including activated T cells, monocytes, fibroblasts and transformed cells. While LIF receptors have been detected on murine monocytes and megakaryocytes, and LIF has been shown to enhance retroviral-mediated gene transfer into bone marrow cells, no effect on normal murine bone marrow cell (BMC) proliferation has been observed. The studies presented here were designed to examine the effects of LIF in combination with other hematopoietic growth factors (HGFs) on unseparated and purified normal BMCs and determine whether these effects were direct or indirect. While LIF had no effect on GM-CSF-induced (previously shown) or IL-3-induced colony formation of normal BMCs, it enhanced CSF-1- and SLF-mediated colony formation. In comparison, LIF enhanced the growth of purified lineage negative (LIN^-) cells in response to GM-CSF, IL-3, CSF-1 and SLF in colony assays. While LIF affected the proliferation of hematopoietic progenitors, it did not affect their differentiation. In addition, these effects were direct since LIF increased the frequency of isolated LIN^- cells that proliferate in response to GM-CSF, IL-3 and CSF-1. We also examined the effects of other members of the LIF receptor family on LIN- cell growth and found that while IL-6 and IL-11 are potent synergistic factors, CNTF-ciliary neurotrophic factor and OSM-oncostatin M have no effects. LIF can directly increase the frequency and size of the more primitive Thy-1^+LIN^- or c-KIT^+LIN^- cells that proliferate in response to SLF plus IL-3. Finally, LIF directly increased the frequency of purified CD-34^+ human progenitors that proliferate in response to GM-CSF or IL-3 plus SLF. Thus, LIF directly promotes the growth of hematopoietic stem/progenitor cells in the presence of other growth factors.

A 210 **THE LIGAND FOR FLT3/FLK2.** Chuck Hannum, Janice Culpepper, David Campbell, Terrill McClanahan, Greg Duda, Sandy Zurawski, Donna Rennick, Janet Wagner, Natalie Martina, Rob Kastelein, Armen Shanafelt, David Peterson, Satish Menon, Warren Dang, Jeanine Mattson, Jeanne Luh, Fernando Bazan, Olivier Rosnet, Patrice Dubreuil, Daniel Birnbaum and Frank Lee. DNAX Research Institute, Palo Alto, CA 94304 and INSERM, 13009 Marseilles, France

Flt3 (also called Flk2) is a receptor tyrosine kinase most homologous to c-kit and c-fms, the receptors for stem cell factor and M-CSF, respectively. Flt3 messenger RNA is found in populations of cells highly enriched for hematopoietic stem cells as well as in progenitor cells. It is also found in fetal liver and brain, and adult brain, thymus, and placenta. The ligand for this receptor is unknown, but it may share similarities with SCF and M-CSF. We will report the purification, sequencing, and partial characterization of the Flt3 ligand. The source of the molecule is the mouse thymic stromal cell line TA4. The purified ligand was digested with endoproteinases, and the resulting peptides were purified by narrow-bore HPLC and individually sequenced. The partial protein sequence defines a novel molecule with the expected biochemical properties of the Flt3 ligand. Biological characterization of the ligand will be presented.

A 211 Molecular evolutionary principles of hematopoietic growth factors

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Previously we demonstrated two molecular evolutionary principles: Development-Related Evolution of CSFs and Concerted Evolution between cytokines and their receptors(Fuchu He & Chutse Wu, Exp Hematol 21:521-524, 1993). Here we analysed molecular evolution of 21 hemopoietic growth factors(HGFs). The result indicated that: (1) at amino acid sequence level, IL-3(multi-CSF) evolved at the highest rates, followed successively by IL-6, IL-4, GM-CSF, IL-9, IL-1α, IL-13, IL-12(p35), IL-2, IL-12(p40), IL-7, M-CSF, IL-5(Eo-CSF), G-CSF, IL-1rα, SCI(MIP-1α), LIF, IL-1β, EPO, IL-11 and SCF(MGF); (2) IL-3, -6 and -4, which have the highest evolutionary rates, evolved at much lower rates in gene sequence, flanking sequence of gene and 5',3' NTRs(nontranslated regions) of cDNA; (3) HGFs evolved at slowing-down rates in species phylogeny, i.e. they evolved faster in lower mammals, much slower in primates and slowest in human. The results showed that the principle of development-related evolution was taken for almost by all the differentiating HGFs(not by proliferating HGFs), and that the regulators of stem cell proliferation might be more important in evolution than differentiating HGFs; implied that the regulating sequences of the genes of those HGFs might play a more important role than their encoding regions; and indicated that HGFs did not follow the "molecular evolution clock".

Tissue Specific Gene Expression (Joint)

D 022 MECHANISMS CONTROLLING KERATINOCYTE-SPECIFIC GENE EXPRESSION: IMPLICATIONS FOR GENE THERAPY AND FOR ELUCIDATING THE GENETIC BASIS OF SKIN DISEASES. Fuchs, E., Aneskievich, B., Byrne, C., Chan, Y.-M., Cheng, J., Faus, I., Hsu, H., Hutton, M.E., Syder, A., Turksen, K., Yang, Z., Zhou, P. and Zinkel, S. Howard Hughes Medical Institute, Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

Keratins are the major structural proteins of keratinocytes. They are coexpressed as specific pairs that form obligatory heteropolymers. When either protein of a pair is mutated within certain domains critical for 10 nm filament assembly, degeneration of the keratinocytes can occur, and this results in genetic disease which is transmitted in an autosomal dominant fashion. Since keratin pairs are differentially expressed during differentiation and development in stratified squamous epithelia, different diseases arise depending upon where the keratin pair is expressed. In addition to their role in degenerative diseases, keratins provide useful biochemical markers to study how complex differentiation programs in epithelia are controlled. A knowledge of the sequences and factors involved in regulating keratin genes should lead to insights into how particular epithelial differentiation fates are controlled during embryonic development. In addition, this information should be valuable in optimizing the promoters-/enhancers of these genes to provide valuable tools for targetting expression of regulatory and structural genes (1) to specific epithelial cells of transgenic mice, for the purpose of generating animal models for human disease, and (2) to keratinocytes in culture, for the purpose of gene therapy. In this talk, I will review what we currently know about genetic disorders of keratin and about keratinocyte-specific gene expression, and discuss how this information can be applied to issues of gene therapy.

D 023 MECHANISMS REGULATING MUSCLE CELL FATE DURING EMBRYOGENESIS, Eric N. Olson, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

Transcription of muscle-specific genes during myogenesis is regulated by combinatorial interactions among cell-type specific and widely expressed transcription factors, and is influenced by extracellular signals that activate complex intracellular pathways of signal transduction. Our laboratory has focused on the mechanisms through which the myogenic helix-loop-helix (HLH) protein myogenin regulates muscle-specific transcription as well as on the mechanisms that regulate transcription of the *myogenin* gene itself. In collaboration with J. Merlie and colleagues (St. Louis), we have generated transgenic mice harboring lacZ transgenes linked to the *myogenin* 5' flanking region. These transgenes recapitulate the complete temporal and spatial pattern of myogenin expression throughout development. The pattern of expression of mutant *myogenin-lacZ* transgenes reveals the existence of heterogeneous myogenic precursor cells that can be distinguished by their endogenous activators of the *myogenin* locus and they suggest that myogenic HLH proteins and the muscle enhancer factor, MEF-2, participate in separate regulatory circuits leading to *myogenin* transcription in the somites and limb buds. In collaboration with P. Hasty, W. Klein and A. Bradley (Houston), the mouse *myogenin* gene has been inactivated by homologous recombination. Mice homozygous for a targeted mutation in the *myogenin* gene survive fetal development but die immediately after birth and exhibit severe reduction of all skeletal muscle. We conclude that myogenin is an essential component of the regulatory pathway leading to skeletal muscle formation during mouse embryogenesis.

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Human Molecular Cytogenetics

D 024 FISH DETECTION OF SUBMICROSCOPIC DELETIONS INVOLVING THE LIS1 GENE IN PATIENTS WITH MILLER-DIEKER SYNDROME AND ISOLATED LISSENCEPHALY SEQUENCE, David H. Ledbetter[1], Romeo Carrozzo[1], Orly Reiner[1], C. Thomas Caskey[1,2], and William B. Dobyns[3], [1]Institute for Molecular Genetics and [2]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, [3]Departments of Neurology and Pediatrics, University of Minnesota Medical School, Minneapolis, MN 55455.

Lissencephaly (agyria-pachygyria) is a human brain malformation manifested by a smooth cerebral surface and abnormal neuronal migration. It can be observed as an isolated abnormality or in association with dysmorphic facial appearance in patients with Miller-Dieker syndrome (MDS). Microdeletions of 17p13.3 are detected in the majority of MDS patients and in an increasing minority of patients with isolated lissencephaly sequence (ILS). A candidate gene, LIS1, has been isolated from fetal brain cDNA libraries which maps to the critical region for lissencephaly and shows non-overlapping deletions in MDS patients. LIS1 shows homology to beta subunits of heterotrimeric G-proteins, suggesting a role in signal transduction pathways crucial for cerebral development. Three cosmid contigs have been developed for FISH analysis corresponding to the 3' region, 5' region, and to probe L132 representing an internal portion of the LIS1 gene. The majority of MDS patients are deleted for the entire LIS1 gene, however, two patients have been identified who are deleted for the 5' region only or the 3' region only. Nine ILS patients have been tested with all three cosmid probe sets by FISH. Five of these patients show no deletion with any probe set. Of the remaining four patients, two are deleted for the 3' region cosmids only, one is deleted for the 3' and L132 cosmids, and one patient has an internal deletion of the L132 cosmids only. An additional twelve ILS patients have been tested with the L132 cosmids and 5' region cosmids. Of these, five patients are not deleted for either probe set, six are deleted for L132 but not for the 5' region, and one patient is deleted for both. Preliminary data indicates the LIS1 gene is large, spanning a genomic distance of >400 kb. Most MDS patients are deleted for the entire gene, while ILS patients tend to have partial gene deletions. In ILS, deletions involve 3' or internal portion of the gene and rarely affect the 5' portion of the gene. More accurate correlation of genotype and phenotype will be possible as the complete genomic region becomes available for deletion mapping in these patients.

Cancer Genetics

D 100 MAPPING OF THREE NON-SMALL CELL LUNG CANCER LOCI TO CHROMOSOME 11, Gerold Bepler, Department of Medicine, Duke University Medical Center, Durham, NC 27710

Mechanisms by which loci for tumor suppressor genes become unmasked are mitotic recombination, deletion, and non-disjunction which result in homozygosity at the respective locus in tumor cells. The size of such loci may be small and elude cytogenetic detection as in rhabdomyosarcoma or large and visible by cytogenetic studies as in small-cell lung cancer. Non-random chromosomal aberrations have been described in non-small cell lung cancer (NSCLC). Their karyotypes are, however, complex and results inconsistent. Therefore, molecular genetic analyses with polymorphic markers may lead to the identification of tumor suppressor genes involved in the pathogenesis of NSCLC. Pairs of specimens (normal tissue/tumor tissue) from 28 previously untreated patients with histologically confirmed NSCLC and from one patient with small-cell lung cancer were obtained immediately after surgical resection. DNA was extracted and analyzed for restriction fragment length polymorphisms (RFLP) and microsatellites using Southern blotting and PCR techniques for 19 markers on chromosome 11. Three distinct loci, one on band 11p13 and two on band 11p15.5, could be identified. The marker *D11S16* on band 11p13 showed loss of heterozygosity (LOH) in 53% (10/19) of cases. The size of the deleted area is approximately 4.6 Mb and does not include *WT1*. *D11S12* on band 15.5 had LOH in 71% (12/17) of cases. This second NSCLC locus is approximately 4.0 Mb in size and bordered by *HBB* and *INS*. A third NSCLC locus is defined by *HRAS* which had LOH in 45% (10/22) of cases. Only one case of small-cell lung cancer was tested which had no LOH on chromosome 11. The total number of NSCLC cases examined so far is too small to allow reliable conclusions on the frequency and distribution of the described NSCLC loci among the various pathological subtypes and clinical outcome groups. Densitometric scanning of autoradiographs for corresponding alleles from *D11S16*, *D11S12*, and *HRAS* showed an approximate doubling of the signal intensity for the remaining allele in 3 of 6 interpretable cases for *D11S16*, 6 of 9 for *D11S12*, and 1 of 5 for *HRAS* which is consistent with genetic duplication. In all other cases LOH appeared to be the result of simple genetic deletion. Simultaneous LOH at different loci in the same tumor was found to be the result from any combination of deletion and duplication, which suggests that LOH at different loci in the same tumor occurs independently and perhaps at different points in time.

D 101 CHARACTERIZATION OF A VARIANT TRANSLOCATION IN A PEDIATRIC APL; MOLECULAR EVIDENCE FOR DYSREGULATION OF *RARA* BY RECOMBINATION WITH A NOVEL LOCUS ON CHROMOSOME 5, Jeff L. Hummel, Richard A. Wells, Suzanne Kamel-Reid and Ian D. Dubé. The Institute of Medical Sciences, the University of Toronto and the University of Toronto Hospitals' Cancer Cytogenetics and Molecular Oncology Program. 100 College Street, Toronto, Ontario, Canada M5G 1L5.

Specific non-random chromosome lesions are closely associated with most human leukemias. Acute promyelocytic leukemia (FAB M3, APL) is associated with a characteristic cytogenetic lesion t(15;17)(q22;q21.1). This translocation results in the juxtaposition of the retinoic acid receptor alpha gene (*RARA*) on chromosome 17 and *PML*, a putative transcriptional regulator on chromosome 15. We describe a pediatric patient who presented clinically with APL, but cytogenetically with a complex karyotype lacking the t(15;17) translocation. Instead, cytogenetic analysis revealed a variant translocation t(5;17)(q35;q21). Southern analysis of patient DNA isolated from bone marrow cells revealed a rearrangement at the *RARA* locus, but not at the *PML* locus, suggesting that *RARA* is dysregulated by the novel recombination with chromosome 5. Using probes specific for the *RARA* gene, the chromosome 17 breakpoint has been narrowed to a 1.2 kb fragment within intron 2 of *RARA*. This breakpoint spanning fragment of normal chromosome 17 has been cloned and partially sequenced. This enabled the synthesis of primers for a one-sided, PCR-based approach to selectively amplify the t(5;17) breakpoint-containing fragment. Currently a 4.4 kb *RARA* restriction fragment containing the region corresponding to the chromosome 17 breakpoint in our patient is being targetted using this technique. Details of this one-sided PCR approach for isolating DNA sequence from the chromosome 5 side of the translocation will be presented, and the potential significance of such a variant translocation in APL will be discussed.

D 102 MOLECULAR CHARACTERIZATION OF A TRANSLOCATION IN A NEUROBLASTOMA-DERIVED CELL LINE INVOLVING THE PROMOTER REGION OF A HOMEOBOX GENE.

Brian D. Lichty, Suzanne Kamel-Reid, Ian Dubé. The Department of Cellular and Molecular Pathology, University of Toronto and The University of Toronto Hospitals' Cancer Cytogenetics and Molecular Oncology Program 100 College Street, Toronto, Ontario, Canada M5G 1L5.

The molecular basis of neuroblastoma is poorly understood. The genes currently thought to play a role in this malignancy most likely contribute to the progression of the disease rather than to its genesis. We are studying a cell line derived from neuroblastoma metastases to the bone marrow of a 4-year old male with stage IV disease. Cytogenetic analysis of this cell line revealed a unique translocation; t(1;10) (p32;q24). We hypothesize that this translocation played a role in oncogenesis via dysregulation of one or more genes at this site. Southern analysis using genomic probes specific for 10q24 demonstrate that the breakpoint in chromosome 10 lies within 450 basepairs containing the promoter region of *HOX11*. This homeobox-encoding gene is often dysregulated in T-cell neoplasms via aberrant recombination with the delta T-cell receptor locus. Northern analysis indicates that the cell line does not express *HOX11*. Southern analyses using genomic probes for candidate genes mapping to chromosome 1p32 such as; *LCK*, *RLF*, *SIL* and *TAL*, revealed no rearrangement in a noncontiguous 135 kb region of 1p32. We are developing a unique one-sided PCR-based approach to amplify a breakpoint-containing fragment using *HOX 11*-specific primers. Our studies represent the first molecular characterization of a translocation breakpoint involving 1p32 in a neuroblastoma cell line. These studies may lead to the cloning of a novel gene from 1p32 involved in the pathogenesis of neuroblastoma.

D 103 Molecular analysis of the breakpoints in AML cells with inv(3)(q21q26), Kazuhiro Morishita, Kazumi Suzukawa, and Jun Yokota. Biology Division, National Cancer Center Research Institute, Tokyo, JAPAN

EVI-1(Ecotropic Viral Integration Site 1) was originally isolated as an oncogene in viral induced murine myeloid leukemias, and is activated in human myeloid leukemias with chromosome 3q26 abnormalities. We analyzed four cases of acute myeloid leukemia cells with inv(3)(q21q26). The EVI-1 gene was expressed in all cases and the chromosomal breakpoints were mapped at about 200 kb downstream of the EVI-1 gene. Breakpoints of all 3q21 region in all insertion cases were mapped within 35 kb of the chromosomal breakpoint of t(3;3)(q21;q26). Thus, it was strongly suggested that in human myeloid leukemias with either t(3;3) or inv(3), the EVI-1 gene is transcriptionally activated by an enhancer element of an unknown gene in near this 3q21 breakpoint cluster area. For this reason, we are currently focusing on the identification of the gene in this area, and identified a transcription unit near the breakpoint.

D 104 FUNCTIONAL ANALYSIS OF DINUCLEOTIDE REPEATS IN AN OVARIAN CANCER CELL LINE,

Kim Orth+, Michael Mathis*, Michael Lovett+#, Anne M. Bowcock#, Mary-Jane Gething^ and Joseph F. Sambrook+#, +Department of Biochemistry, *Department of Pathology, #McDermott Center for Human Development, and ^Howard Hughes Medical Institute, University of Texas Southwestern Medical School, Dallas, TX 75235

Replication errors (RER) occurring in a number of di- and tri-nucleotide repeats throughout the genome have been linked to genetic disorders (i.e. Huntington's Syndrome, Fragile X Syndrome, Myotonic Dystrophy) and have been associated with colon and ovarian cancers. In each of these disorders, errors in the replication/repair machinery lead to the expansion or contraction of these repeated sequences. PCR analysis of genomic DNA isolated from the ovarian serous cystadenocarcinoma #2774 cell line reveals a large number of alleles (>10) at any one $(TG)_n$ microsatellite. However, cytogenetic analysis of these cells shows that they are primarily diploid or triploid. Clonal cell lines (n=10) were derived and PCR analysis revealed that any one clonal cell line encoded only 2-3 alleles at any one locus. We are expanding these clonal lines and after successive passages are testing them for any changes in the number of repeats at different loci. In addition, we have designed a functional assay to test the fidelity of the replication machinery. We plan to use this assay to isolate factors that may be altered in the replication/repair machinery.

D 105 CLONING OF A NEW MEMBER OF THE BETA-ADAPTIN GENE FAMILY FROM HUMAN CHROMOSOME 22 : A CANDIDATE GENE INVOLVED IN MENINGIOMA TUMORIGENESIS,

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Meningiomas are human solid tumors of the meninges covering the central nervous system which are found either solely or along with other tumor types in Neurofibromatosis type 2. Cytogenetic and molecular genetic studies of meningiomas pointed to a high frequency of monosomy 22 in the tumor cells (up to 70%). Southern Blot analysis of the chromosome with DNA probes showed the involvement of the long arm (22q) in at least four regions deleted in a number of tumor cases. At present, two of these candidate regions are being analysed through the exon-amplification method, enabling us to extract transcriptionally active DNA sequences out of the cosmid contig covering these portions of the chromosome. From a region of 22q12 around the marker D22S342, which was homozygously deleted in one tumor case, we cloned a new member of the β-adaptin gene family. This gene constitutes therefore a candidate for being involved *in the* development of meningioma. The β-adaptins are proteins of cytoplasmic heterotetrameric complexes called adaptors. These play an important role in the endocytosis process by allowing receptor-ligand complexes to be internalized into clathrin-coated vesicles. Further investigation such as point mutation screening and Northern blotting analysis are being performed in order to confirm or reject the role of the β-adaptin gene in the development of meningioma.

D 106 CLONING OF A PUTATIVE MENINGIOMA TUMOR SUPPRESSOR GENE,

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In meningioma abberations of chromosome 22 occur in approximately 65% of the cases. These abberations include loss of one copy of the chromosome, interstitial deletions and translocations. They suggest that a tumor suppressor gene on chromosome 22 is involved in the pathogenesis of meningioma. We have cloned a gene, MN1, which is located on chromosome 22 and which is disrupted by a reciprocal translocation in a meningioma. This gene is distinct from the recently cloned NF-2 gene. The MN1 gene consisits of two large exons interrupted by an intron of approximately 60 kb. The translocation breakpoint was found to be in the 5'exon and presumably is in the open reading frame. In cells cultured from the tumor in which the reciprocal translocation was observed, the other allele of the MN1 gene is physically intact, as judged from southern blots. However no expression of the gene is observed using northern blots. In other meningiomas the expression of the MN1 gene is highly variable, ranging from not detectable to a very high expression. These results suggest that the MN1 gene could be a tumor suppressor gene which is important for the development of meningiomas.

D 107 THE MOLECULAR CHARACTERIZATION OF A LOW PENETRANCE RETINOBLASTOMA PEDIGREE,

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The pediatric ocular tumor retinoblastoma is a paradigm of the two hit hypothesis model of recessive oncogenesis. In familial retinoblastoma, the inherited mutation predisposes all retinal cells to cancer while a second somatic mutation is required for actual tumorigenesis. The penetrance of bilateral retinoblastoma is usually high, however in a small but significant number of families the penetrance of the disease is reduced substantially. Molecular genetic study of such families should identify partially functional RB1 alleles and help clarify the role of such mutations on the RB1 protein *in vivo*. In one large pedigree with low penetrance retinoblastoma, a putative cryptic splicing mutation has been identified which lies outside the standard splicing consensus sequence. This results in the elimination of exon 21 from the cDNA. Studies of cell lines with functionally similar mutations indicate that this protein, if expressed *in vivo*, would be nonfunctional. The reduced level of mutant, as compared to wild type, cDNA suggests that this mutation affects the frequency of correct splicing at exon 21, resulting *in a decreased* but not absent level of WT RB1 mRNA. The corresponding reduced level of protein may account for the partial penetrance of disease in this family.

D 108 *NF2* GENE MUTATIONS IN MENINGIOMAS AND VESTIBULAR SCHWANNOMAS

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The gene for the hereditary disease neurofibromatosis 2 (NF2), which predisposes for benign central nervous system tumors such as vestibular Schwannomas and meningiomas, has been assigned to chromosome 22 and has recently been isolated. We have used reverse transcriptase-PCR amplification followed by SSCP and DNA sequence analysis to screen for mutations in the *NF2* gene in sporadic meningiomas and vestibular Schwannomas. Inactivating mutations caused by deletions, insertions and point mutations were found in approximately one third of the cases. Of the 15 tumors in which a mutation in the *NF2* gene was observed and the copy number of chromosome 22 could be established, 12 also showed LOH for the relevant region of chromosome 22. This suggests that also in the sporadic tumors the *NF2* gene functions as a genuine tumor suppressor gene. The mutations found mostly resulted in frameshifts, leading to truncation whithin the N-terminal half of the putative protein. Therefore, it seems that the N-terminal part of the gene either forms a hot spot for mutations, or that only mutations in this region of the protein truly result in a loss-of-function.

Gene Targeting and Transgenesis

D 200 IN-OUT GENE TARGETING CONSTRUCTS FOR A MOUSE MODEL OF β-AMYLOID DEPOSITION.

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Six mutations at four locations within or close to the β-amyloid region of the amyloid precursor protein (APP) gene have been linked to diseases involving premature deposition of β-amyloid, including Alzheimer's disease (AD) and hereditary cerebral hemorrhage with amyloidosis-dutch type (HCHWA-D). The production of animals carrying these mutations may facilitate the elucidation of the sequence of events leading to early deposition of β-amyloid in affected individuals and would provide a model for testing the efficacy of new drugs. However aged mice do not normally show β-amyloid deposition and the affect of the three amino acid differences between human and mouse β-amyloid on it's deposition in mice also requires investigation. Constructs making use of the in-out gene targeting strategy have been engineered containing a 3Kb HPRT minigene for +/- selection and a 6.8Kb KpnI genomic fragment of mouse APP into which the three base pair changes needed to 'humanise' mouse β-amyloid have been introduced using p-Alter mutagenesis. The Alzheimer linked 717 val to ile and HCHWA-D 693 glu to gln mutations have also been introduced by PCR mutagenesis with or without the 'humanization' changes. There is a unique XbaI site within the KpnI fragment for linearization of the constructs prior to electoporation into HPRT$^-$ E14 embryonic stem (ES) cells. Following selection targeted ES cell lines containing the mutations and/or the 'humanization' changes can be identified by SSCA.

D 201 A METHOD FOR REPEATEDLY TARGETING GENES IN MURINE EMBRYONIC STEM (ES) CELLS

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We have developed a method allowing the repeated targeting of any gene or gene complex. Normally, the targeting of a gene requires the screening of hundreds to thousands of ES cell clones to identify those that are correctly targeted. When many different targeting events at the same locus are desired, the screening process must be repeated for each modification. We have developed a two step method of targeting that eliminates screening for all but the first modification. In the first step, a truncated (promoterless) HPRT gene is integrated near the gene of interest in an ES cell line lacking endogenous HPRT function. The second step modifies the murine gene and completes the previously inserted HPRT gene. The intact HPRT gene allows direct selection of targeted clones with aminopterin.

We have successfully used this system to delete a 20 kb region of the mouse genome containing both adult ß-globin genes. From forty million cells we obtained one HAT resistant colony. Southern analysis of DNA from this colony confirmed the deletion. Chimeric mice have been produced from this ES cell strain by blastocyst injection. The next alteration will replace the adult mouse ß-globin genes with human $ß^S$-globin which is responsible for sickle cell anemia.

D 202 GENE TARGETING IN NORMAL, DIPLOID HUMAN CELLS, Robert B. DuBridge, Fred C. Ousley, Steven R. Williams, Fariba Dai and Mitchell H. Finer, Cell Genesys Inc., 322 Lakeside Dr., Foster City, CA 94404

Homologous recombination (gene targeting) is a powerful tool which has only recently become available for the manipulation of mammalian genomes. To date, use of this procedure has been reported for the modification of mouse ES cells and transformed/immortalized mammalian cell lines. Our goal is to create therapeutically useful products based on normal, diploid human cells. To generalize the therapeutic potential of cellular products to a wide variety of unrelated patients, the use of gene targeting has to be applied to normal human cells to remove barriers to allogeneic cellular transplantation, namely the genes controlling the major histocompatability complex (MHC) antigens. To this end, the effectiveness of gene targeting in normal human retinal pigmented epithelial (RPE) cells has been demonstrated. Primary human RPE cells were isolated from human cadaver donors using the procedure of Meyerson *et al.* Using a vector which contained 5Kb of homology to the human HPRT locus with an insertion of a neo^r marker in exon 3, both insertional as well as replacement recombination events were demonstrated in the single HPRT gene in RPE cells derived from a young, male donor. Both types of recombination events occurred with a frequency of approximately 10^{-7} recombinants per input cell. These recombinants were identified using a G418 + 6-thioguanine selection procedure. The clones were expanded and verified by Southern analysis. Intramolecular recombination was studied in this system by examining the reversion frequency of several of the insertional mutants. All of these mutants reverted to an $HPRT^+$, neo^s phenotype at an equivalent rate. These revertants, which were detected at a frequency of 10^{-6} per input cell, were shown to be true recombinants by Southern analysis.

Subsequent experiments demonstrated the feasibility of targeting loci relevant for the cell-surface expression of class I MHC antigens. Gene targeting at the β_2-microglobulin locus in hRPE cells was achieved with high efficiency using a targeting vector which created a novel fusion protein upon homologous recombination. Accurately targeted clones were detected at a frequency approaching 10^{-5} recombinants/input cell using an ELISA-based assay system . Targeted clones were expanded and confirmed, both by immunoprecipitation of the correct fusion protein and by conventional Southern analysis. These results demonstrate the feasibility of using gene targeting to precisely engineer normal cells to combat human disease.

D 203 TARGETED INACTIVATION OF THE MOUSE Muc-1 GENE LOCUS, A GENE CODING FOR A CARCINOMA-ASSOCIATED MUCIN S.J. Gendler, A.P. Spicer,V.M.M. Braga, M. Wilson and S. Savarirayan, SC Johnson Medical Research Center, Mayo Clinic, 13400 E.Shea Blvd, Scottsdale, AZ 85259

MUC1 is a heavily glycosylated membrane mucin glycoprotein which is (a) developmentally regulated and (b) highly expressed and aberrantly glycosylated by the majority of carcinomas and in particular, by >92% of primary and metastatic breast cancers. The gene is expressed in most simple secretory epithelial cells in both the developing embryo and adult. Muc-1 expression correlates with epithelial differentiation in the stomach, pancreas, lung, trachea, kidney, endometrium, salivary and mammary glands, where it lines the apical surfaces of the developing lumens. The gene encodes an integral membrane protein with a large O-glycosylated extracellular domain which extends far above the apical cell surface. It is hypothesized that the high level of expression of the MUC1 protein by carcinoma cells may confer an advantage upon the cell, perhaps by reducing the adhesive properties and/or by modulating the immunogenicity of the tumor cells. It is also thought that, during development, expression at the apical surfaces of simple epithelial tissues may block adhesion molecules and aid in the formation of lumens. To test these hypotheses, we have mutated the mouse Muc-1 locus in ES cells using homologous recombination. In the construct designed for deletion of the gene, the lacZ reporter gene was fused in frame with the Muc-1 promoter, thereby allowing expression of this fusion construct to be followed by staining for ß-galactosidase activity. The cells which would normally express the Muc-1 gene will stain positively for ß-galactosidase. The mutated ES cells were injected into 3.5 day blastocysts which were reimplanted into pseudopregnant mice. The frequency of targeted replacement of the Muc-1 gene was found to be approximately 1 in 18 antibiotic resistant colonies. Chimeric mice have been obtained which exhibit germline transmission; these mice are being bred for homozygosity. The developmental effect of deleting the Muc-1 protein in embryos at different stages of development will be presented. Since mammary gland cancer in the mouse conserves many of the features of the human disease, tumors will be generated in mutant and control mice and analyzed for growth, differentiation, immunological characteristics and metastatic potential. If Muc-1 is found to confer an advantage to tumor cells, then it may be possible to down-modulate the Muc-1 gene, possibly producing tumor cells that are more susceptible to NK or T cell killing or exhibiting decreased metastatic properties.

D 204 PROGRESS TOWARD GENERATING TYPE 1 GAUCHER MICE BY INTRODUCTION OF THE N370S POINT MUTATION BY HOMOLOGOUS RECOMBINATION INTO ES CELLS, E.I. Ginns, H. Yoshikawa, C.E. McKinney, B.K. Stubblefield, S. Winfield, L. Carmon, B.M. Martin, R. Willemsen[1], E. Sidransky, and M.E. LaMarca, Clin. Neurosci. Br., NIMH, NIH, Bethesda, MD 20892. [1]Erasmus Univ., Rotterdam, Netherlands

Gaucher disease, the deficiency of the enzyme glucocerebrosidase, is the most common lysosomal storage disorder and sphingolipidosis. As a result of this enzyme deficiency, glucocerebroside accumulates and is stored predominantly within reticuloendothelial cells. While at least 30 mutations have been identified in the glucocerebrosidase gene, the point mutation N370S in exon 9 is most commonly encountered and is often associated with mild disease. A transgenic mouse strain homozygous for a null allele was previously generated by targeted disruption of the murine glucocerebrosidase gene. These mice die within 24 hours of birth and have a phenotype that is analogous to severely affected neonates with type 2 Gaucher disease. We have now introduced the mutation resulting in the substitution of asparagine for serine (N370S) into the mouse glucocerebrosidase gene and inserted this DNA into a targeting plasmid, pPNT, containing neomycin resistance and HSV-tk. This point mutation was introduced into murine embryonic stem cells (J1 line, R. Jaenisch) and these genetically altered cells were injected into C57/B6 blastocysts to generate chimeric mice carrying the point mutation. Chimeric mice with >90% agouti coat color carrying the N370S mutation are being bred with mice heterozygous for the null glucocerebrosidase allele in order to generate mice with a N370S/null genotype. It is anticipated that mice with the N370S/N370S or the N370S/null genotype will have a milder phenotype more similar to that of type 1 Gaucher patients.

D 205 CONSTRUCTION AND ANALYSIS OF p53-DEFICIENT MICE BY A NOVEL GENE REPLACEMENT SYSTEM: EVIDENCE FOR THE LOSS OF HETEROZYGOSITY OF THE p53 GENE IN TUMORS, Yoichi Gondo, Kenji Nakamura, Kazuki Nakao, Toshikuni Sasaoka, Motoya Katsuki, Laboratory of Embryonic and Genetic Engineering, Medical Institute of Bioregulation, Kyushu University 69, Fukuoka 812, JAPAN

We developed a novel gene replacement method by using two steps of homologous recombination. First, we targeted one allele of the p53 gene of mouse ES cells. We used a targeting vector containing a cassette of the neo^R and HSV-tk genes between two p53 genomic sequences. The targeted cells were selected with G418. Then we replaced this targeted allele with the second targeting vector and by the GANC selection. With this method, we isolated an ES cell line, in which almost all of the p53 coding sequences were replaced to that of the ß-galactosidase (lacZ) gene in frame. The established ES cell line does not carry any selectable marker genes, the neo^R and HSV-tk genes, after all. In spite of two steps of gene targeting and many passages, the ES cells are still able to transmit the replaced allele to the offspring through germ-line chimeras. Homozygotes as well as a germ-line chimera and heterozygotes carrying the replaced p53 allele have developed tumors. The Southern blot analysis of the chimera and heterozygotes has so far revealed that the wild-type allele of the p53 gene in tumors was disappeared. This is the first direct evidence for the loss of the heterozygosity of the p53 gene associated with tumorigenesis.

D 206 CREATION OF A MURINE MODEL OF FUMARYLACETOACTETATE DEFICIENCY, Markus Grompe*, Muhsen Al-Dhalimy*, Nancy Kennaway*, Milton Finegold** and Phillipe Soriano**, *Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR 97201; **Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Tyrosinemia type I is an autosomal recessive metabolic disease caused by deficiency of fumarylacetoacetate hydrolase (FAH), the last step of tyrosine catabolism. Its main clinical features include progressive liver failure, renal tubular damage and the development of liver cancer. In order to create an animal model of this disorder for the testing of new dietary, pharmacological and genetic therapies, we have recently generated mice deficient in FAH by homologous recombination in embryonic stem cells.

At birth 25% of pups derived from heterozygous parents are homozygous for the gene disruption, indicating that it is not lethal in utero. However, all homozygote animals die within the first 24 h after birth and suffer from severe hypoglycemia. FAH enzyme activity in the liver was completely absent. Quantitative plasma amino acid analysis revealed an increased phenylalanine/tyrosine ratio in the affected animals.

Recently it was discovered that FAH and a gene of interest to developmental biologists, the hepatocyte specific developmental regulation locus (hsdr-1), both lie on the same 300 kb fragment within the 3.8 Mb X-ray induced deletion of the c^{14CoS} albino mutant mouse. Mice homozygous for this deletion fail to express several different liver specific genes and die as neonates. This finding raises the possibility that FAH is identical to hsdr-1. Northern blot analysis of hsdr-1 dependent mRNAs in livers of the knock-out mice showed a pattern identical to that seen in c^{14CoS} homozygotes. Additional phenotypic and biochemical analysis provides evidence that the FAH and hsdr-1 loci are indeed identical.

D 207 HUMAN Cu-Zn SUPEROXIDE DISMUTASE TRANSGENIC FVB/N MICE: TOOLS FOR STUDYING SOME HUMAN DISEASES, *London J, Casanova M., Toyama K., Paly E., Paris D., Sinet P.M.* CNRS URA 1335,Hôpital Necker, 149 rue de Sèvres 75743 Paris Cedex 15 - France.

As an approach to the development of an animal model for Down Syndrome, we have generated a human CuZn Superoxide Dismutase (hSOD-1) transgenic line in the FVB/N inbred mouse strain allowing to have transgenic progeny with the same genetic background. The heterozygous hSOD-1 mice obtained carry 12 to 15 copies of the transgene. The transgene expression is ubiquitous and very high in brain, thymus and erythrocytes (4 to 8 fold). By Northern analysis of various tissues, two hSOD-1 mRNAs are visualized at 0.6 and 0.9 kb ; their relative intensities vary according to tissues tested. In all tissues except in the brain the 0.6 kb band is predominant. Electrophoretic and quantitative analysis of the SOD-1 protein allows us to show that in most tissues the transgene expression seems regulated similarly to the murine gene except in liver and kidney. Moreover we show that in blood the murine and the transgenic SOD-1 levels are decreasing with age. Similar studies are now in progress for other tissues especially brain and thymus in which a premature involution is suggested by preliminary results.

This transgenic line might also contribute to the study of Parkinson's disease as we have shown that surexpression of hSOD-1 prevents the destruction of nigrostriatal dopaminergic neurons following administration of MPTP, a drug used for modeling Parkinson disease. All the results obtained on the regulation of SOD-1 expression in heterozygous and homozygous hSOD-1 transgenic mice might also be interesting for the study of the ALS syndrome in which an altered SOD-1 gene has been demonstrated.

D 208 APPLICATION OF GENE TARGETING IN ES CELLS AND TRANSGENESIS TO ESTABLISH MOUSE MODELS FOR INHERITED HUMAN SKIN DISORDERS, Thomas M Magin*, Harry Navsaria+, I Leigh+ and David W Melton*, Institute of Cell and Molecular Biology, The University of Edinburgh, Mayfield Road, Edinburgh*; and The Department of Experimental Dermatology, London Hospital Medical College, Ashfield Street, London+

Cytokeratins (CKs), which comprise two biochemically and immunologically distinct subgroups, are the most complex class of the intermediate filament (IF) multigene family. At least one member of each CK subgroup is expressed in any given epithelium to form heteropolymeric CK filaments and different epithelia are characterised by different CK patterns. The basal layer of epidermis expresses the CK pair 5/14 which upon differentiation is gradually replaced by the expression of CKs 1/10 and others. The recent discovery of point mutations in several CK genes expressed in human epidermis has for the first time hinted at a function for IF in tissue stability. Single amino acid alterations in CKs seem critically involved in the human genetic skin disorders epidermolysis bullosa simplex (EBS) and epidermolytic hyperkeratosis. The dominant inheritance pattern of most disease variants seems to be a direct consequence of the assembly properties and architecture of IF. The position of point mutations in a given CK and its level of expression might be diagnostic for both the nature of disease and its severity.

To study in greater detail the role of CKs in various aspects of these blistering skin disorders we have extended our experience in gene targeting in mouse ES cells to CK genes. These models will be used to evaluate somatic gene therapy approaches. To this end we introduced an intact IF subunit in keratinocytes isolated from an EBS patient and into transgenic mice in order to complement the defective IF cytokeratins.

D 209 *HOXA-2* MUTANT MICE EXHIBIT HOMEOTIC TRANSFORMATION OF SKELETAL ELEMENTS DERIVED FROM CRANIAL NEURAL CREST, Maureen Gendron-Maguire, Moisés Mallo, Maobin Zhang[1] and Thomas Gridley[2], Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110, USA, and [1]Department of Toxicology and Pathology, Hoffmann-LaRoche Inc., Nutley, NJ 07110, USA

Mice homozygous for a targeted mutation of the *Hoxa-2* (*Hox 1.11*) gene die within 24 hours of birth, exhibiting obvious malformations of the external ear. Analysis of stained skeletons revealed that homozygous mutant animals contained multiple cranial skeletal defects, including a duplication of the ossification centers of the bones of the middle ear. Histological analysis showed that this duplication resulted from the transformation of skeletal elements derived from the second branchial arch into more anterior structures, resulting in a duplication of Meckel's cartilage in the region of the otic capsule. Homozygous mutant animals also exhibited defects in the formation of cranial nerves IX and X. These data provide direct experimental evidence for the existence of a branchial *Hox* code.

D 210 TARGETED DISRUPTION OF THE METALLOTHIONEIN I AND II GENES IN MOUSE, Anna E. Michalska and K.H. Andy Choo, Murdoch Institute, Royal Children's Hospital, Parkville, Victoria 3052, Australia

Metallothioneins (MTs) are small, cysteine-rich proteins that bind heavy metals such as Cd, Zn, Cu and Hg. They are thought to be involved in homeostasis and availability of zinc and copper within cell, heavy metal detoxification, and in host-defence processes. In addition, it has been suggested that MTs may also be involved in aspects of normal growth and development. To gain further insight into the roles of these proteins we have generated mice that are unable to produce MT-I and MT-II.

We have utilised homologous recombination to introduce mutations into the MT-I and MT-II loci of mouse embryonic stem (ES) cells. Chimeric mice resulting from the targeted ES cells transmitted the disrupted alleles through their germline. Homozygous animals were born alive, and appeared phenotypically normal and fertile. Absence of MT-I and MT-II proteins was confirmed by direct measurement in liver extracts. Challenging the mutant animals with moderate levels of $CdSO_4$ indicated their greater susceptibility to cadmium toxicity than wild type animals. These mice should provide a useful model to allow detailed study of the physiological roles of MT-I and MT-II.

D 211 TARGETED DISRUPTION OF THE MURINE STROMELYSIN-1 (MMP3) GENE, John S. Mudgett, Nicole A. Chartrain, Howard Chen, Myrna Trumbauer, Michael Tocci, and Nancy I. Hutchinson. Department of Molecular Immunology, Merck Research Laboratories, Rahway, NJ 07065.

Murine stromelysin-1 (MMP3) is a member of the matrix metalloproteinase family implicated in tissue remodeling in development, repair, and in disease processes. We constructed cosmid libraries from mouse embryonic stem (ES) cell genomic DNA, isolated the murine stromelysin-1 (SLN-1) gene, and constructed replacement vectors to inactivate the catalytic domain of the stromelysin-1 gene in mouse ES cells. We used a dual approach to isolate SLN-1 targeted ES cells: 1) isolating individual transformants and characterizing their genomic DNAs, and 2) culturing small pools of about 15 transformants and analyzing the pools' DNA by PCR. All PCR positive ES cell lines were fully characterized by Southern hybridization to confirm targeted disruption of the stromelysin gene. Independent SLN-1 targeted ES cell lines were injected into mouse blastocysts, and progeny mice were screened for coat color chimerism. Male coat color chimeric mice were bred for determination of germline penetrance and generation of heterozygous SLN-$1^{-/+}$ offspring in both C57BL/6 and 129Sv mouse lines. Viable homozygous stromelysin-1 knock-out mice were generated, and Northern and western hybridization analysis was used to demonstrate the disrupted stromelysin-1 gene was not functional. No obvious phenotypic differences were observed between the knock-out and wild-type animals, and all male and female homozygous mice tested were fertile. The disrupted stromelysin-1 gene is being bred into mouse strains susceptible to induced arthritis, and the role of stromelysin-1 in experimental arthritis will be assessed.

D 212 EXPRESSION OF A DOMINANT NEGATIVE MUTANT OF PDGF IN TRANSGENIC MICE CAUSES IMPLANTATION FAILURE, Marcela Pekna, Milos Pekny, Arne Östman, Karin Forsberg, Richardo Feinstein, Carl-Henrik Heldin, Christer Betsholtz, Bengt Westermark; Dept. of Pathology, University Hospital, 751 85 Uppsala, Sweden

Platelet-derived growth factor (PDGF) is a potent growth factor for a variety of cells. The active ligand is made up as a dimer of A and B chains that are covalently linked by disulfide bonds. All three isoforms of PDGF have been identified, i.e. PDGF-AA, PDGF-AB and PDGF-BB. Two types of PDGF receptors have been identified, the alpha-receptor that binds all isoforms with equally high affinities, and the beta-receptor that only binds PDGF-BB with high affinity. PDGF is a bivalent molecule; one chain in PDGF binds one receptor molecule resulting in receptor dimerization that is required for the activation of the protein-tyrosine kinase of the receptor. The finding that the v-sis oncogene of simian sarcoma virus (SSV) is a transduced PDGF B chain gene shows that PDGF has oncogenic potential. Despite a lot of in vitro data, the function of PDGF in vivo remains poorly understood.

To attenuate endogenous expression of PDGF in vivo we used a vector containing a metallothionein promoter-driven PDGF-A mutant which *in vitro* does not bind to the receptor but can dimerize with the endogenous native PDGF chains thereby titrating them out. Transgenic mice were generated by pronucleus injections using standard techniques. High expression of the transgene was found in 3 out of 4 transgenic lines studied. Protein production and secretion was confirmed by ELISA on conditioned media from cultures of cells from transgenic E13-14 embryos.

The only pathological finding was infertility of transgenic females apparently caused by **implantation failure**. At the time implantation normally occurs, we find absence of decidual transformation of the uterus (a prerequisite for the implantation), very low serum progesterone levels and absence of corpora lutea. Embryo-transfer studies have shown that if early embryos (one-cell stage or blastocyst stage) were transferred from transgenic females to foster mothers, they normally developed to term. This finding suggests a crucial role for the PDGF family during the early pregnancy.

D 213 DISRUPTION OF GLIAL FIBRILLARY ACIDIC PROTEIN GENE IN ES CELLS BY HOMOLOGOUS RECOMBINATION, Milos Pekny, Per Levéen, Bengt Westermark, Christer Betsholtz; Dept. of Pathology, University Hospital, S 751 85 Uppsala, Sweden.

Glial fibrillary acidic protein (GFAP) is the main component of the intermediate filaments of the cytoskeleton in astrocytes. GFAP synthesis is influenced by many factors, including age, neural trauma, inflammation and hypoxia. GFAP is phosphorylated by cdc2 kinase and other kinase(s) on serine and threonine residues at different time points during mitosis, the impact of which remains unknown. GFAP is present in many intracranial or intraspinal tumors of glial origin.

To evaluate the involvement of GFAP in the development of CNS we have attempted to create a null mutation of a GFAP gene in mouse via homologous recombination in embryonic stem cells (ES cells).

A targeting vector prepared using 12 kpb of genomic DNA and with the promoter region replaced by a neomycin cassette was transfected into 129 OLA E14 ES cells. These were maintained on neomycin resistent primary mouse embryonic feeder cells in the presence of LIF. Following G418 selection, ES cell clones were tested using Southern blot analysis (the frequency of a homologous recombination event was 3/90). Two ES cell clones with a copy of the GFAP gene disrupted were used for blastocyst injection. Several chimeras have been produced and are currently being bred.

D 214 IGF2 TRANSGENIC MICE PRODUCED BY INJECTING AN IN VITRO METHYLATED GENE, Dimitrina D. Pravtcheva and Thomas L. Wise, Pediatric Research Institute, Saint Louis University Medical Center, Saint Louis, MO *63110*

The insulin-like growth factor -2 (Igf2) gene is believed to be involved in the control of embryonic growth. In both mice and humans the Igf2 gene is imprinted, and is expressed only when inherited from the father. The growth stimulatory effect and the imprinting pattern of the Igf2 gene, as well as its location on human chromosome 11p15.5 have led to the suggestion that increased dosage of IGF2 is responsible for the fetal overgrowth observed in the Beckwith-Wiedemann syndrome (BWS). Direct proof of the role of IGF2 in the growth abnormalities and tumor predisposition in BWS would require the production of animals that overexpress Igf2. However, efforts to produce Igf2 transgenic mice have so far been *unsuccessful, presumably* because of a lethal effect of Igf2 on the injected embryos. We sought to achieve a reversible suppression of Igf2 activity and avoid its deleterious effects on the injected embryos by in vitro methylation of Igf2 genomic DNA prior to injection. Because of the dynamic changes in methylation in germ cell and embryo development, we hypothesized that the Igf2 transgenes may undergo partial or complete demethylation and reactivation in the transgenic founders or their progeny. We injected an ~ 35 kb genomic fragment containing the entire mouse Igf2 gene, with 8 kb of 5' and 15 kb of 3' flanking sequences. The injected clone was in vitro methylated with SssI methyltransferase. Two out of ten mice that developed from the injected embryos had retained the injected Igf2 gene. Southern analysis of progeny of these founders indicated that one of them most likely contains three separate transgene loci. Preliminary analysis of the methylation pattern of the transgene in the founders indicated that the transgenes retained an overall high degree of methylation, at least in the tail. Analysis of e14 and e15 embryos has so far indicated a lower degree of transgene methylation in the F1 embryos, compared with the founder. Northern analysis of embryo and yolk sac RNA from these embryos showed no obvious quantitative differences in the amount of Igf2 transcripts between transgenic and non-transgenic littermates. A more detailed analysis of the methylation differences between the transgenes and the endogenous Igf2 genes is currently underway. We are also investigating the effect of repeated passage of the transgene through the germ line, on its methylation and expression.

D 215 HOMOLOGOUS RECOMBINATION IN MOUSE EMBRYONIC STEM CELLS WITH AN ABSOLUTE FREQUENCY OF 10^{-1}, Nancy Smyth Templeton and Brian Safer, Molecular Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

The frequency of homologous recombination per electroporated cell is defined as the absolute frequency. This frequency in mouse embryonic stem (ES) cells is extremely low, approximately 10^{-6}, by current procedures. We have developed a method for gene targeting in mouse ES cells that produces an absolute frequency of 10^{-1}. This method will be presented at the meeting. Because the absolute frequency is extremely high, it should be possible to perform gene targeting without the use of selectable markers and use of homologous recombination for gene therapy becomes possible. The latter is valuable because homologous recombination can allow precise targeted insertion of genetic information, gene expression in appropriate tissues at appropriate levels, correction of mutations including dominant mutations, and gene inactivation. Presently, we are performing homologous recombination in a variety of human cells and cell lines. Plans are underway for delivery of targeting DNAs to correct specific defects in somatic tissues of animals or to eliminate harmful sequences by gene inactivation.

D 216 MURINE MODEL OF FAMILIAL DEFECTIVE APOLIPOPROTEIN B100 BY TARGETED MODIFICATION OF THE APOLIPOPROTEIN B GENE, Lori Toth and Nobuyo Maeda, Department of Pathology and Curriculum in Genetics, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7525

A correlation between high levels of low density lipoprotein (LDL) in plasma and premature coronary heart disease is well established. LDL is cleared from plasma via receptor-mediated endocytosis by the LDL receptor. Apolipoprotein B100 (apoB100) is virtually the only protein component of LDL and functions as the ligand for the LDL receptor. Genetic defects in the apoB100 protein that interfere with or abolish receptor-ligand interaction cause the human disorder familial defective apolipoprotein B100 (FDB). FDB is characterized by increased plasma concentrations of apoB100, cholesterol, and LDL and high risk for developing atherosclerosis. We are using an "in-out" targeting method in murine embryonic stem cells to generate mice with subtle site-specific apoB100 mutations that result in protein unable to bind to the LDL receptor. The "in step", a homologous integration reaction, disrupts apoB coding sequence and generates a duplication of the target locus. The "in step" introduces a premature stop codon into exon sequence yielding a truncated apoB protein. We have chimeric mice harbouring this genetic change and are breeding for transmission of the modified gene. Mice with this mutation are expected to be models of familial hypobetalipoproteinemia. During the "out-step", recombination between the duplicated repeats generated in the integration reaction removes the duplication and all exogenous sequence except for modifications in the region of putative LDL receptor binding that abolish receptor-ligand interaction. We have obtained correctly modified embryonic stem cell lines and are in the process of generating mice with this altered apoB100 allele. These animals are expected to be deficient in receptor-mediated endocytosis of LDL particles from plasma resulting in accumulation of atherogenic LDL in the circulation. Such animals may be at high risk for developing atherosclerotic cardiovascular disease and model FDB in humans.

D 217 The Mouse Strain Mpv 17, A Disease Model Linking Glomerulosclerosis To Loss Of A Peroxisomal Function.

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Mice homozygous for a retroviral insertion at the Mpv 17 locus develop glomerulosclerosis and nephrotic syndrome at early age. The disrupted gene, the Mpv17 gene, is therefore a recessive glomerulosclerosis gene in mice (Cell 62, 425). It codes for a deduced protein of 176 aa that contains two or more possible transmembrane regions and shows striking homology to the peroxisomal membrane protein pmp 22. Indeed, immunolocalisation of the Mpv 17 protein product using antibodies against bacterially derived GST-Mpv 17 fusion protein revealed peroxisomal localisation of the Mpv 17 protein. Thus, the Mpv 17 strain discloses a link between the loss of a peroxisomal function and glomerulosclerosis in mice.

To study the potential role of a human homolog in disease we isolated this gene and investigated its structure and function. The conservation between the murine and the human gene appears to be more than 90% and in both organisms the gene is single copy. This suggests that mutations in this gene may have serious consequences in humans as well. By cloning and analysis of the human genomic Mpv 17 region we established a PCR protocol to isolate the human Mpv 17 coding region from blood DNA (Hum. Mol. Genet., 2, in press). At present, we study the Mpv 17 gene in several patients suffering from hereditary kidney disease.

We are attempting to rescue the phenotype of the Mpv 17 mice by transgenesis. Hopefully, this will also reveal new insights into expression targetting of genes important for kidney and liver function.

In summary, the Mpv 17 mouse strain may serve as a valuable animal model for glomerulosclerosis and its possible treatment in general and for inherited forms of this disease in particular.

Mutational Analysis

D 300 MUTATION ANALYSIS OF THE FANCONI ANEMIA GENE *FACC*. Arleen D. Auerbach, Jean D. Lin, Masako U. Udono, Qiao Zhang, Peter C. Verlander. The Rockefeller University, New York, NY 10021

Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive disorder defined by hypersensitivity of cells to DNA cross-linking agents; a gene for complementation group C *(FACC)* has recently been cloned. In order to identify mutations for carrier screening, as well as to obtain a better estimate of the fraction of FA patients in group C, 174 racially and ethnically diverse families in the International Fanconi Anemia Registry (IFAR) were examined by single strand conformation polymorphism (SSCP) analysis for mutations in *FACC*. Six mutations were identified that cosegregate with the disease phenotype, including two that have not been previously described, while two polymorphisms were found that apparently are not disease-associated. We have identified 14 families in which affected individuals are homozygotes for *FACC*, 3 families in which affected individuals are compound heterozygotes, and 8 families in which only a single allele is known. Thus, mutations were found in a total of 25 of 174 families screened (14.4%). The most frequent mutations were IVS4+4 A→T (intron 4) and 322delG (exon 1), which are present in 88% of the families assigned thus far to group C. Other less common mutations include Q13X (exon 1), R185X and D195V (exon 6), and L554P (exon 14). The polymorphisms were S26F (exon 1) and G139E (exon 4). In order to examine genotype-phenotype correlations in patients with known mutations in *FACC*, a scoring system based on phenotype characteristics was applied (Auerbach et al, 1989, Blood 73:391); the simplified score is proportional to the severity of the phenotype. Patients with IVS4+4 A→T have a very severe phenotype, with multiple congenital malformations and early onset of hematologic disease (mean simplified score=4.7) All of these patients are of Jewish ancestry and are homozygous for this mutation. All but one patient with mutations in exon 1 or 6 have a very mild form of the disease, with no major birth defects (mean simplified score=2.3). Ancestry of these patients is Northern or Eastern European (322delG, R185X, D195V) or Southern Italian (Q13X). The only patient with a severe phenotype in this group is homozygous for 322delG. The two brothers with an exon 14 mutation, of Northern European ancestry, have a severe phenotype. We have recently developed amplification refractory mutation system (ARMS) assays for rapid screening for *FACC* mutations; we are applying these assays to determine the frequency of the common *FACC* mutations in appropriate populations and to identify carriers in *FACC* families.

D 301 MULTIPLE NEURAL ALTERNATIVE SPLICE PRODUCTS OF OCRL-1, THE GENE CAUSING THE OCULO-CEREBRO-RENAL SYNDROME OF LOWE. Lawrence Charnas, Denise Rodney, and Robert L. Nussbaum Human Genetics Branch NICHD, Bethesda, MD, 20892 and Dept. of Human Genetics, Univ of Pennsylvania School of Medicine, 19104.

The Oculocerebrorenal Syndrome of Lowe (OCRL) is an X-linked, recessive disorder, characterized by congenital cataracts, cognitive dysfunction, and renal tubular Fanconi syndrome. A 5.8 kb transcript for OCRL-1 has been seen in most human and mouse tissue. A 1 kb mouse fibroblast cDNA, lMFI17, contains bases 1741-2782 of the mouse OCRL transcript and is 90% identical to the human sequence. A 24-bp insertion at nucleotide 2352 encoding the peptide sequence EDSYLEKE was found in all 11 distinct OCRL cDNA clones isolated from a mouse brain library and all three OCRL cDNA from a human fetal brain library that included this region. This insertion occurs at a known exon-intron boundary in both the mouse and human genome. In PC12 cells, a rat pheochromocytoma and sympathetic neuron precursor cell line, two different transcripts, 3.1 and 4.4 kb, were detected. Four unique cDNAs were isolated after screening 2 million clones of a PC12 cell library. Partial sequence from one clone shows 98% identity to mouse cDNA from nucleotide position 1470 -2350 with a unique inframe sequence at the 5' site of the brain specific exon insertion. This suggests that OCRL-1 has multiple, organ neural specific isoforms and may have implications for understanding the pleiotropic effects of the syndrome.

D 302 MUTATIONS IN KERATIN-RELATED GENO-DERMATOSES ARE CLUSTERED IN MOLECULAR OVERLAP REGIONS OF KERATIN INTERMEDIATE FILAMENTS. C. Chipev, J.-M. Yang, J. DiGiovanna, S. Bale, J. Compton and P. Steinert, Skin Biology Branch, NIAMS, NIH, Bethesda, MD 20892.

By use of crosslinking experiments, we have determined the axial alignments of the coiled-coil molecules in human K1/K10 and K5/K14 keratin filaments. In building a two-dimensional surface lattice model, we found that the last 5-10 residues of the rod domain segment 2B of one molecule overlap the first 5-10 residues of the 1A rod domain segment of a similarly-directed molecule. This means that several sequence regions of the keratin chains overlap each other: H1, begining of 1A, end of 2B and H2. These four sequence regions represent the most conserved throughout the families of keratin chains, presumably because they specify molecular overlaps. Coincident with these studies, we have determined the likely mutations in either the K1 or K10 chain of 11 probands of epidermolytic hyperkeratosis, a disease that affects the organization of the keratin filaments of the supra-basal layers of the epidermis. When this body of data is combined with that from other laboratories for another disease, epidermolysis bullosa simplex, which involves mutations of the K5 and K14 chains of the basal layer of the epidermis, several features become evident. Of the 19 different positions that are mutated in these two diseases, many occur in the same residue positions in the various chains. Furthermore, 85% of the changes occur in the H1, 1A and 2B sequence regions of the overlap described above, with most occurring in the 1A portion. This non-random distribution of mutations for the first time provides a systematic basis for understanding the structural implications of these geno-dermatoses. The inappropriate amino acid substitutions caused by the mutations presumably significantly affect the structure and dynamic function of the filaments in the epidermis. The observation of clustering will facilitate further identification of mutations, because workers will now know where to look. This data base will aid in genetic counselling of patients at risk for disease, and provide a focus for future gene therapy strategies for treatment of the diseases.

D 303 ALLELE SPECIFIC MULTIPLEX PCR DETECTING COMMON CRYSTIC FIBROSIS AND TAY-SACHS DISEASE MUTATIONS IN ASHKENAZI JEWS, James J. Dermody, Department of Microbiology and the Center for Human and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, New Jersey 07103.

Cystic fibrosis and Tay-Sachs disease occur at high frequency in the Ashkenazi Jewish population with carrier frequencies of approximately 1/25. Four CF mutations and three Tay-Sachs mutations account for greater than 95% of the respective disease alleles observed in this population. Allele specific PCR conditions have been formulated that allow simultaneous amplification of the common mutations in each disease. The analysis is completed by visual inspection of a single lane on an ethidium bromide stained polyacrylamide gel. This assay provides an efficient means of carrier screening in populations which harbor a small number of mutations constituting the majority of defective alleles for a particular disease.

D 304 MOLECULAR PATHOGENESIS AND STEPS TOWARDS THE THERAPY OF A LYSOSOMAL STORAGE DISEASE, Anu Jalanko, Aija Riikonen, Nina Enomaa, Kai Tenhunen, Annukka Isoniemi, Minna Peltola and Leena Peltonen, Department of Human Molecular Genetics, National Public Health Institute, Helsinki, FIN 00300; Finland.

Apartylglucosaminuria (AGU) is a lysosomal storage disease due to mutations in the aspartylglucosaminidase (AGA) gene. This disease is strongly enriched in the Finnish population where the carrier frequency is 1:40.

We have isolated the AGA cDNA and clarified the individual steps in the intracellular biosynthesis and processing of the enzyme. In Finland 98 % of AGU alleles contain a double mutation resulting in Cys_{163} -> Ser and Arg_{161} - > Gln substitutions. We have analysed the consequences of these mutations expressing them separately in vitro and shown that the Cys_{163} -> Ser mutation is responsible of the deficient enzyme activity. One disulfide bond is missing resulting in early intracellular degradation of the enzyme (Ikonen et al., EMBO J. 10: 51, 1991; Genomics 11: 206, 1991; EMBO J. 12: 295, 1993).

Opposite to Finland, each AGU family outside Scandinavia has carried a different unique mutation. We have characterized twelve different mutations of the coding region (Ikonen et al., PNAS 88: 11222, 1991) and found three mutations affecting the regulatory motifs in the 5' and 3' noncoding regions.

We have initiated enzyme and gene replacement studies using AGU fibroblasts as a preliminary target. Complete correction of the enzyme activity was obtained with recombinant AGA secreted from CHO cells. The steps taken towards the animal model of AGU include the cloning of the mouse AGA gene and construction of ES cell lines carrying a knock out mutation mimicking the disease.

These experiments will allow gene replacement studies and finally lead to the understanding of the tissue-specific changes in the disease.

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D 305 CHARACTERIZATION OF ARGININOSUCCINATE SYNTHETASE MUTATIONS IN CITRULLINEMIA FIBROBLAST AC, Chi-Ming Li[1] and Tsung-Sheng Su[1,2], [1]Graduate Institute of Genetics, National Yang-Ming Medical College and [2]Departmant of Medical Research, Veterans General Hospital-Taipei, Taiwan, Republic of China.

Citrullinemia is an autosomal recessive disorder resulting from the deficiency of argininosuccinate synthetase. To elucidate the molecular mechanism involved in this disorder, a cultured skin fibroblast established from a citrullinemia patient, AC, is chosen for this study. Previous study by S1 nuclease analysis of argininosuccinate synthetase RNA in this fibroblast has revealed two types of mutations: one responsible for S1 nuclease-detectable abnormal RNA, another leading to no detectable mRNA, i.e. RNA negative. By DNA sequence analysis of genomic DNA amplified through polymerase chain reaction, a single base substitution within the splice acceptor site of intron 6 is shown to be responsible for S1 nuclease-detectable RNA abnormality. This mutation leads to splicing defect by skipping entire exon 7 sequences. Besides, a minor species of RNA utilizing a cryptic splice acceptor site site on exon 7 is also demonstrated. To study the defect in RNA-negative allele, the precursor RNA level from allelic argininosuccinate synthetase genes of citrullinemia fibroblast AC was examined. The results from this study suggest that the mutation that responsible for decreasing level of mature RNA does not exert its effect through transcription initiation. Rather, a defect in a post-transcriptional event at or prior to RNA polyadenylation may be involved.

D 306 IDENTIFICATION OF MUTATIONS IN THE INTERLEUKIN-2 RECEPTOR GAMMA CHAIN GENE (IL2RG) ASSOCIATED WITH X-LINKED SEVERE COMBINED IMMUNODEFICIENCY. Jennifer Puck, Xingge Liu, Paula Henthorn, Children's Hosp of Phila and U of PA Sch of Med and Vet Med, Phila., PA 19104.

The γ chain of the lymphocyte receptor for interleukin-2, IL2RG, must be expressed in order for IL-2, the major stimulatory T-cell cytokine, to be internalized and to cause cell activation. IL2RG lies in Xq13.1 and is mutated in the X-linked form of severe combined immunodeficiency, SCIDX1, in which affected males have impaired lymphocyte maturation. Originally published mutations included 4 nonsense and 3 missense mutations (Noguchi, Cell 73:147, 1993; Puck, Hum Mol Genet 2:1099, 1993). The 4.2 kb IL2RG gene has 8 exons encoding a 1.8 kb mRNA. Primers flanking each exon were constructed to permit PCR of 260-290 bp sections from genomic DNA to screen for conformational variations in unrelated males with SCID. SSCP was performed using end-labeled primers and pre-amplified genomic templates from SCID patients and their family members as controls. Denatured products were separated on MDE gels and autoradiographed. Abnormal migration patterns were detected in 15 patients without adjusting electrophoresis conditions: exon 1 (1 patient), 2 (2 patients), 3 (2 patients), 4 (5 patients) and 5 (5 patients), all in extracellular domains. No SSCP abnormalities were detected in exons 6, 7 or 8, encoding the transmembrane and intracellular domains, raising the possibility that missense mutations in exons 6-8 are not associated with the SCID phenotype. Direct sequencing of the PCR-amplified exons with altered SSCP patterns in each case documented a unique mutation, often confirmed in family members. These included 2-bp and 6-bp deletions in exon 5 and an array of single base changes producing stop codons or amino acid substitutions. Newly arising mutations were demonstrated at the sequence level in 2 families, confirming previous genetic analyses consisting of X chromosome inactivation assay of female carriers' T lymphocytes and linkage analysis.

D 307 A SPECIFIC HAPLOTYPE ON CHROMOSOME 1q21-q31 DEFINES THE LOCUS OF JUVENILE OPEN ANGLE GLAUCOMA IN A LARGE FRENCH-CANADIAN PEDIGREE, V. Raymond[1, 2], M. Plante[2], G. Côté[3], J.-L. Anctil[3], J. Dugré[3], J. Weissenbach[4], C. Laberge[2] and J. Morissette[2], [1]Endocrinologie moléculaire et [2]ontogénèse et génétique moléculaire, Centre de recherche du CHUL, Québec, Qué, G1V 4G2, Canada; [3]Département d'ophtalmologie, Hôpital du St-Sacrement, Québec, Qué. Canada; [4]Genethon, BP 60, 91002 Evry cedex, France

Primary open angle glaucoma (POAG) is an eye disorder characterized by elevated intraocular pressure and an optic neuropathy causing progressive loss of visual fields. When uncontrolled, POAG leads to blindness. Juvenile open angle glaucoma (JOAG) is a subclass of POAG that appears before the age of forty. In the few pedigrees described, JOAG was found to be inherited in an autosomal dominant fashion. The etiology of POAG is unknown.

One of us (G.C.) has previously described a very large French-Canadian family highly affected by JOAG (Côté et al. Canad. J. Ophthal. 3: 331, 1968). More than 200 members over 4 generations have been investigated. In this large family, the mode of JOAG inheritance was autosomal dominant. A founder effect was highly suspected. In order to identify the disease-causing gene, we thus initiated an haplotype study. Microsatellite markers in the 1q21-q31 region were selected since linkage of JOAG to this region was recently observed by Sheffield et al. (Nature Genetics 4: 47-50, 1993). Four AFM markers located between D1S218 and D1S212 were typed in 13 patients and 22 normal individuals. In 11 of these 13 patients a characteristic haplotype was clearly recognized. One of the two other patients had a different form of glaucoma while the other affected individual may demonstrate a recombination event. Out of the 22 normal persons, two individuals show the characteristic haplotype and are at risk of developing JOAG. These results thus further confirm the localization of juvenile open angle glaucoma to the 1q21-q31 region and provide this French-Canadian family with a new diagnostic tool to identify the at risk individuals.

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D 308 *X-LINKED AGAMMAGLOBULINEMIA* (XLA), A CANDIDATE DISEASE FOR GENE THERAPY.

C. I. Edvard Smith, Igor Vorechovsky, Paschalis Sideras, Berivan Baskin, Lennart Hammarström, Lars Brandén, Susanne Müller, Kleanthis G. Xanthopoulos and David Vetrie.
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X-linked agammaglobulinemia (XLA) is characterized by a B-lymphocyte differentiation defect causing agammaglobulinemia and increased susceptibility to infections in affected males. We have recently used a positional cloning strategy, based on cDNA enrichment on yeast artificial chromosomes, for the isolation of this gene (*Nature* 361:226, 1993) and in a collaborative study demonstrated that the same gene is mutated in the mouse strain *xid* (*Science* 261:355, 1993). The gene was found to encode a novel protein-tyrosine kinase and was named *Btk* (formerly *Atk* or *Bpk*). In order to further characterize XLA we have investigated the expression pattern of the *Btk* gene at the level of mRNA and protein. The gene is expressed in most hematopoietic cells, including mast cells and progenitor cells expressing the early differentiation marker CD34, and is specifically down-regulated in T lymphocytes and plasma cells. We are presently carrying out several studies, including mutation analysis, and characterization of the flanking genes, isolated by the selection procedure, and these data will be presented. We are also constructing retroviral vectors for experimental transfer of the gene. XLA belongs to the rare group of disorders having a differentiation block, making it an interesting candidate for gene therapy as corrected cells are expected to selectively mature.

Techniques and Disease Identification

D 400 MOLECULAR CLONING OF POTENTIAL TRIPLET REPEAT EXPANSION TARGETS

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Expansion of triplet repeat sequences in human genes is recognized as an important type of mutagenesis leading to disease. The recent discoveries of expanded trinucleotide repeats being responsible for Fragile X syndrome (FRAX), myotonic dystrophy (DM), spino-bulbar muscular atrophy (SBMA), Huntington's disease (HD), and spinocerebellar ataxia type 1 (SCA1), has attracted significant research interest. Previous work has shown the existence of 400 to 500 repetitive elements of the sequence $(CAG/A)_n$ in the genome of *Drosophila melanogaster*. This is the same repeat that is expanded in SBMA, HD, and SCA1. Additionally, a search of Genbank reveals that this repeat is present in over twenty other human genes. This data suggests the existence of other human genes that contain triplet repeats of the sequence $(CAG)_n$. In order to examine this hypothesis we have utilized a PCR based method for isolating fragments of cDNA's from the glioma cell line NAT and from human brain that contain repeats of the sequence $(CAG)_n$. Data will be presented indicating the existence of previously uncharacterized neural cDNA's containing triplet repeats that could be potential targets for expansion.

D 401 INTRACELLULAR FATE OF DNA MOLECULES DELIVERED INTO B LYMPHOCYTES BY pH-INSENSITIVE LIPOSOMES, Kenneth Crook and Lee Leserman; Centre d'Immunologie de Marseille-Luminy, Parc Scientifique de Luminy 13288 Marseille cedex 9, France.

We are currently investigating the potential of pH-insensitive liposomes as delivery systems for larger DNA molecules, as, up until now, they have been overshadowed by viral vectors and cationic liposomes which have taken the lead in gene therapy protocols. The problem with these liposomes is that they travel all the way to the lysosomes before releasing their contents, thus putting the latter at risk of degradation. However, we have already shown that pH-insensitive liposomes can deliver a wide variety of reagents (proteins, drugs, oligonucleotides) to lymphocytes, suggesting that this degradation is not completely refractory to a functional effect. We have been interested in examining the intracellular fate of encapsulated reagent following receptor-mediated endocytosis. To do this we have encapsulated a marked cDNA molecule encoding a marker gene inside small (0.1-0.2μm) liposomes and coupled hen-egg lysozyme or Protein A to their surface. These liposomes are then targeted specifically to the surface immunoglobulin of B cells from mice transgenic for anti-HEL IgM. Subcellular fractionation at various times following endocytosis will allow us to determine the localisation of the DNA molecule, as well as estimate its approximate half-life within the cells. Analysis of mRNA and protein will tell us whether the gene has been properly transcribed and translated. We believe the results will have significance for optimisation of gene delivery for eventual gene therapy considerations.

D 402 CHARACTERIZATION OF CLINICAL CASES USING LABELLED COSMID PROBES, Barbara R. DuPont[1], Robert F. Stratton[1], Ann Fertitta[2], Anne S. Olsen[2], Stephen G. Ryan[1], and Charleen M. Moore[1], (1) The University of Texas Health Science Center at San Antonio, TX 78284 (2) Lawrence Livermore National Laboratory, Livermore, CA 94551

De novo chromosome rearrangements and abnormalities have often been difficult to characterize by conventional cytogenetic banding techniques. The use of fluorescent *in-situ* hybridization (FISH) as a diagnostic aid in clinical cytogenetics has become widespread. We have recently used cosmid probes specific for chromosome 19p to characterize two unrelated cases. Each case presented with unusual lower facial features including vertical ridging on the chin. Initial karyotypes were determined to be normal in both cases, but high resolution chromosome analysis later revealed a subtle 19p+ in one. Cosmid probes specific for 19p12 (14683), 19p13.1 (15734), and 19p13.2 (16922, 15423, 21246, 20259) were used to define the extra short arm material as a direct duplication of bands 19p13.1, with duplication of probe 15734, and part of 19p13.2, with duplication of probe 16922. No *duplications* or deletions of this region were observed in the second case using these same cosmid probes, although similarities seen in the facial features of these two cases suggested such a duplication. We have also used cosmids specific for chromosome 5q31-5q33 to characterize this region in an unusual case. The patient presented with the typical features of Treacher Collins syndrome and also had significant short stature and microcephaly. High resolution chromosome analysis failed to reveal any abnormalities. FISH analysis using probes specific for the Treacher Collins region, NXN6 (117B2) and RPS14 (129F2, 47H8), failed to detect any deletion within this region which would have explained the combination of features seen in this patient. We are continuing to use labelled cosmid probes to characterize clinical cases with unusual features in order to detect rare contiguous gene syndromes.

D 403 PCR ANALYSIS USING CAPILLARY ELECTROPHORESIS. Sandy M. Koepf, Barnett B. Rosenblum, Frank Oaks, Michael Wenz, Dave Yamane, Sandy L. Spurgeon, and Bill Efcavitch. Applied Biosystems Division of Perkin-Elmer, 850 Lincoln Centre Dr., Foster City, CA 94404.

For a majority of PCR applications, determining the size of a PCR fragment, as well as the relative amount, is necessary. Traditionally, agarose and/or polyacrylamide slab gels have been used to determine this information. Although considered easy to use, slab gels are labor intensive and can involve handling hazardous chemicals. We have focused on developing an entangled polymer which allows the analysis of PCR fragments to be carried out using capillary electrophoresis (CE). There are many advantages to using CE. Automation, minimal sample amounts, and reproducibility are just a few. The properties of the polymer allow the PCR fragments in the size range of less than 100 bp to over 1 Kb to be analyzed accurately. In some cases, the results obtained with CE are more informative than those obtained with traditional slab gels. For this reason, analysis with CE is useful for a wide range of PCR applications including analysis of templates for PCR sequencing.

D 404 MOLECULAR DEFECTS IN HLA CLASS II NEGATIVE CONGENITAL IMMUNODEFICIENCY Janet S. Lee, Chitra Saraiya, Gregory Bannish, Mary Susan Brady, Grace Ungers, and Clifford Hume, Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY 10021.

Absence of HLA class II antigens on B lymphocytes is the basis of a category of congenital immunodeficiencies (CID). We have shown that mutations responsible for lack of expression in CID cell lines fall into three complementation groups, with a fourth group represented by a similar mutant derived in vitro. Our studies further indicated that the genetic defects for each group in HLA class II expression map outside the HLA class II region. Transient expression experiments using a small fragment of the HLA DRA promoter demonstrate that the mutant cell lines are all defective at the level of transcription of HLA class II genes. In an effort to identify defects that might be associated with the immunodeficiency, we examined DNA binding proteins in nuclear extracts from mutant B cell lines and identified one complex (C1) that is reduced or absent when compared to those in normal lines. All others, including a complex called RF-X, that was previously thought to account for the defect, were normal. Nuclear extracts from two complementation groups fail to transcribe a DRA promoter construct in vitro, reflecting their DRA negative phenotypes. Finally, though cell lines from different groups complement each other in vivo, no complementation was observed by mixing extracts for transcription in vitro. Experiments are in progress to identify and characterize the gene that is defective in complementation group I.

D 405 Normalized blood glucose in *db/db* transgenic mice with overexpression of human Glucose Transporter 4.
John McNeish, Jeff Stock, Scott McCoid, Jeff Pessin*, A.J. Milici and E. Michael Gibbs. Pfizer Central Research, Groton, CT. 06340, *University of Iowa

We have examined the physiologic role of the human muscle/fat specific facilitative glucose transporter (GLUT 4) in transgenic mice derived on the diabetic mutant, inbred strain C57Bl/6 *db/db*. Previously, hybrid transgenic mice expressing this transgene demonstrated marked reduction in oral glucose tolerance tests (OGTT) versus nontransgenic mice (Liu et al. PNAS in press). These transgenic mice also have reduced serum insulin, increased basal glucose uptake in isolated adipocytes and elevated levels of cell surface associated GLUT 4. These results indicate that constitutively increased expression of the human GLUT 4 protein results in efficient metabolic control of plasma glucose fluctuations *in vivo*. The murine *db/db* mutation phenotype is similar to Human Type II diabetes, as well as being obese and sterile. To test the effects of increased human GLUT 4 in diabetic condition, transgenic mice were produced on the C57Bl/6 *db/db* inbred, mutant background. All overexpressing lines examined by OGTT as *db/+* heterozygotes have reduced plasma glucose levels following glucose challenge compared to age matched nontransgenic controls. Currently, three expressing lines have been bred to homozygosity at the *db* locus and subjected to OGTT. The nontransgenic *db/db* mice have a fasting plasma glucose of ~200 mg/dl. with an increase to ~600-700 mg/dl. 30 minutes post oral glucose challenge at 1.0 mg/kg body weight. The transgenic *db/db* mice have 4-fold lower fasting glucose concentration (~50 mg/dl) which interestingly rises only slightly after glucose challenge (~70-90 mg/dl), representing ~10 fold reduction versus nontransgenic *db/db* mice. Other physiologic parameters including serum insulin, triglycerides, free fatty acid concentration, and subcellular GLUT 4 localization are being examined and will be discussed.

D 406 DEVELOPMENT OF LARGE HUMAN ARTIFICIAL EPISOMAL CHROMOSOMES (HAECs), Tian-Qiang Sun[1] and Jean-Michel Vos [1,2,1] Department of Biochemistry and Biophysics, [2]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, NC 27599-7295.

A human system, based on the latent replication origin oriP of Epstein-Barr virus, has been developed for propagating up to 330 kb exogenous DNA inserts in human cells as human artificial episomal chromosomes (HAECs). The human inserts in various individual HAEC clones are derived from different regions of the human genome and appear to represent faithful replicas of the source DNA. No structural change is detected on the HAECs after 60 generations of cell growth. If is expected that the oriP not only serves as the replication origin, but also is involved in partitioning replicated DNA molecules into daughter cells during cell division. This autologous HAEC system provides an experimental approach for cloning, mapping, and functional analysis of large human DNA regions in human cells.

D 407 THE PAX6 PROTEIN FUNCTIONS AS A TRANSCRIPTION ACTIVATOR, Kejun Tang and Grady F. Saunders, Department of Biochemistry and Molecular Biology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Aniridia is a developmental disorder characterized by complete or partial absence of the iris. The gene responsible for aniridia, PAX6, has been cloned by our lab and others recently. The predicted protein product of PAX6 revealed 2 DNA binding motifs, a paired domain and a paired-type homeodomain, both are conserved in the PAX gene family. Structural characteristics suggest that PAX6 may function as a transcriptional regulator in the development of the eye, however, whether it is a transcription activator or repressor is unknown. Here we present evidence that the C-terminal 150 amino acid sequence of PAX6, when fused to the GAL4 DNA binding domain, is capable of activating transcription of a heterologous promoter containing UAS_G. Our results suggest that the molecular role of PAX6 could be a transcription activator that activates the expression of several downstream genes which specify the formation of particular eye structures such as the iris. This result is consistant with the fact that the null phenotype of PAX6 shows the loss of certain craniofacial structures including the eyes and nose rather than overgrowth or transformation.

D 408 THE RESOURCE FOR MOLECULAR CYTOGENETICS AT THE LAWRENCE BERKELEY LABORATORY AND THE UNIVERSITY OF CALIFORNIA, SAN FRANCISCO. Heinz-Ulrich Weier, Colin Collins, Wen-Lin Kuo, Damir Sudar, Daniel Pinkel and Joe W. Gray, Div. Mol. Cytometry, Dept. Laboratory Medicine, University of California, San Francisco, CA94143-0808.

The Resource for Molecular Cytogenetics is a newly founded research group within the Life Sciences Division at the Lawrence Berkeley Lab. and the Department of Laboratory Medicine, University of California, San Francisco.

The goals of the resource are:

1. to provide the scientific community with physically mapped large insert [≈100 kb] DNA probes spaced in 5-10 Mb intervals throughout the whole human genome;

2. generate and distribute DNA repeat probes and high complexity, chromosome-specific DNA libraries for all human and selected rodent chromosomes;

3. further develop hybridization techniques such as comparative genomic hybridization (CGH); and

4. develop instrumentation for computer-assisted and automated molecular cytogenetic analysis.

In this presentation, we show the various strategies applied for probe isolation, our first results using P1 clones for fluorescence in situ hybridization (FISH) and discuss availability of probes. We will also summarize the current capabilities for digital image analysis.

The Resource can be reached via Internet at remoc@dmc.ucsf.edu.

D 409 FULL LENGTH HUMAN DYSTROPHIN cDNA EXPRESSION IN mdx MOUSE BY HVJ-LIPOSOME METHOD Itaru Yanagihara* , Yasufumi Kaneda+ , George Dickson# , Koji Inui* and Shintaro Okada*, *Department of Pediatrics,Osaka University Faculty of Medicine, 2-2 Yamada-oka, Suita-City, Osaka 565, Japan, +Institute for Molecularbiology, 1-3 Yamada-oka, Suita-City, Osaka 565, Japan, #Department of Experimental Pathology, U.M.D.S., Guy's Hospital, London Bridge, London SE1 9RT, United Kingdom

Duchenne Muscular Dystrophy (DMD) is X-linked muscular dystrophy, which occurs 1 in 3500 male birth.Most of the patients died of respiratory or heart failure around the second decade of their life.Pathological findings of muscle are caused by a defect of muscle cytoskeleton called dystrophin, a 427KDa protein encoded by a 14kbp transcript. Myoblast transfer and gene therapy has been proposed to correct the deficiency of dystrophin.

Here, we report high efficacy of gene expression of full length human dystrophin gene in mdx mouse muscle in vivo by HVJ-liposome method. We have constructed 3 expression vectors driven from Rous sarcoma virus promotor (pRSV-Dy), mouse leukemia virus promptor (pDMD8) and natural human muscle dystrophin promotor (pDyDy). Each vector was coincubated with non-histone HMG1 (high mobility group 1) protein to make plasmid protein complex. The plasmid-HMG1 complex was then encapsuled by vortex method into liposome prepared by phosphatidylserine, phosphatidylcholine and cholesterol. The liposome was then mixed with inactivated HVJ (Sendai virus) to fascilitate fusion with cell membranes.

The prepared HVJ-liposome was directly injected into 12-~18- week-old mdx mice quadriceps muscle. On day 3, 5 and 10 after injection , dystrophin expression was detected by immunofluorescene analysis using 3 kinds of monoclonal (N,C-terminus and rod domain) and polyclonal antibodies. Dystrophin was expressed as a ring-like pattern in the cytoplasm and beneath the muscle membrane. In the study of the liposome containing 20 μg of pRSV-Dy vector, ring-like staning pattern was observed 16% (52/324) of the myofibers and muscle membrane pattern was observed 26% (84/324) of the myofibers at maximum 3 days after injection The dystrophin expression was detected until 10 days but tended to decrease. The most efficient vector was pRSV-Dy, although lessor expression of dystrophin was observed in pDMD8 and pDyDy. Without HMG1 protein, no dystrophin expression was observed.

With HVJ-liposome method, we have succeeded to express full length human dystrophin in vivo mdx mouse muscle. This gene transfer system enabled transient expression of dystrophin for about 2 weeks. Furthter study will be necessary to express the transgene for a longer period.

Gene Mapping

D 500 GENETIC AND PHYSICAL MAPPING OF THE GENE FOR FAMILIAL MEDITERRANEAN FEVER (FMF), I. Aksentijevich[1], E. Levy[1], E. Pras[1], X. Chen[1], L. Ferrin[2], T. Keith[3], M. Pras[4], and D.L. Kastner[1], [1]ARB, NIAMS, and [2]GBB, NIDDK, NIH, Bethesda, MD; [3]Collaborative Research, Inc., Waltham, MA; [4]Heller Institute for Medical Research, Sheba Medical Center, Tel Hashomer, Israel

Familial Mediterranean fever (FMF) is a recessively inherited disorder characterized by attacks of fever and serositis; patients may also develop systemic amyloidosis. We recently mapped the gene causing FMF (designated "*MEF*") to chromosome 16p. In order to define the location of *MEF* more precisely, we genotyped 51 non-Ashkenazi Jewish FMF families (324 individuals, 162 affected) for 11 markers on 16p. Our data placed *MEF* between *D16S63* (CRI-0327) and *D16S246* (218EP6), a genetic interval of approximately 3 cM. Using an $(AC)_n$ polymorphism from the 0327 cosmid, we identified 4 recombinants at the centromeric end of the *MEF* interval. There was only one recombinant that defined the telomeric end, but this was based on a less informative RFLP. For 3 loci telomeric to *D16S246*, we have observed strong linkage disequilibrium among Moroccan but not non-Moroccan Jewish families, probably reflecting a founder effect. At *D16S246* we observed substantial linkage disequilibrium in both groups, consistent with the greater proximity of *MEF* to this locus. In order to estimate the physical distance between these flanking markers, we are using RecA-assisted restriction endonuclease cleavage of DNA (RARE). This technique can be used to excise a high molecular weight genomic fragment between well-defined EcoRI sites within the flanking markers. The size of such a fragment can be determined using PFGE.

D 501 MOLECULAR GENETIC CHARACTERIZATION OF THE DEAF *SNELL'S WALTZER* MOUSE, Karen B. Avraham, David M. Kingsley,* Liane B. Russell,† Neal G. Copeland and Nancy A. Jenkins. Mammalian Genetics Laboratory, ABL-BRP, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702; *Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305; †Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

Mouse mutations offer an invaluable tool for isolating disease genes by positional cloning. The *Snell's waltzer (sv)* mutation exhibits deafness and movement disorders. A study of the *sv* gene may provide insight into the molecular mechanisms underlying sensorineural hearing impairment in humans. The *sv* locus maps 2 cM distal to the *dilute* and *short ear* loci on chromosome 9. A number of radiation-induced mutations have previously been isolated that affect one or more genes in this region. One of these mutations, se^{sv}, produces a viable, short eared, Snell's waltzer phenotype when homozygous. Previous genetic studies (Russell, 1971, *Mutation Res.*, 11: 107-123) suggest that this mutation is a chromosome inversion with one endpoint in or near the *short ear* gene and the other in or near the *sv* gene. Molecular probes from the *short ear* region detect altered restriction fragments in DNA from mice carrying the se^{sv} mutation. We have cloned the altered restriction fragments, sequenced the changes, and shown that the mutation does indeed consist of a chromosome inversion with one end near the *short ear* gene. New probes from the other side of the inversion map ~2 cM distal of the *short ear* gene and provide the first molecular access to the *sv* region. Attempts are currently underway to identify the *sv* gene by methods such as exon trapping and cDNA screening.

In addition, a physical map of the *d-se* region is being made using Yeast Artificial Chromosomes (YACs) to construct a contig of this region, which will further facilitate analysis of the *sv* locus. This contig will also provide molecular access to other genetically defined, developmentally important genes in this region of chromosome 9.

Research sponsored by the National Cancer Institute, DHHS, under contract number NO1-CO-74101 with ABL.

D 502 MAPPING IN THE REGION OF THE MACROPHAGE RESISTANCE GENE *Lsh/Ity/Bcg* ON MOUSE CHROMOSOME 1 AND FURTHER ANALYSIS OF THE CANDIDATE *Nramp*. Anne-Marie Baker, C. Howard Barton, Simon H. Whitehead and Jenefer M. Blackwell, University of Cambridge Clinical School, Department of Medicine, Addenbrooke's Hospital, Cambridge, CB2 2QQ, U.K.

Resistance to the macrophage pathogens *Leishmania donovani*, *Salmonella typhimurium* and *Mycobacterium bovis* is controlled by a single gene (*Lsh/Ity/Bcg*) located on the proximal region of mouse chromosome 1. Recently Vidal and coworkers (*Cell* **73**:469-485, 1993) employed a positional cloning strategy to identify a candidate for *Lsh/Ity/Bcg*, designated *Nramp* (Natural resistance associated macrophage protein). In pursuing a similar strategy we have identified several new yeast artificial chromosome (YAC)-derived markers in the region which have been mapped with respect to known markers (*Cryg, Creb-1, Vil, and Pax-3*) using a panel of backcross mice made between chromosome 1 congenic mouse strains. This has permitted a clearer definition of the genetic boundaries for C57L-derived chromosome 1 material in N10 and N20 B10.L-*Lsh* congenic mouse strains. Screening of a cDNA library made from activated bone marrow-derived macrophages from N10 B10.L-*Lsh* mice yielded a series of clones which differed in the 5' region from the published (Vidal *et al.*, *ibid*) pre-B cell-derived clone sequence, resulting in the addition of 64 amino acids at the N-terminus of the predicted protein. YACs from the region carry the sequence for this long-form of the *Nramp* gene, which we have demonstrated is the form expressed in the cell-type (activated macrophages) responsible for the resistance phenotype. Sequence analysis reveals the presence of a putative SH3 signal transduction domain, consistent with the many functional studies demonstrating the role of *Lsh/Ity/Bcg* in regulating the priming/activation of macrophages. Retroviral constructs bearing the long-form *Nramp* molecules have been generated and are currently being analysed for their ability to transfer the resistance phenotype both *in vivo* and *in vitro*.

D 503 THE HOLT-ORAM SYNDROME GENE MAPS TO HUMAN CHROMOSOME 12q2, Craig T. Basson, Glenn S. Cowley, Scott Solomon, Barbara Weissman, Andrew K. Poznanski, Thomas A. Traill, J.G. Seidman, Christine E. Seidman, Cardiovascular Division, Dept. of Medicine, Brigham and Women's Hospital, Boston, MA 02115

Holt-Oram Syndrome is an autosomal dominant condition with variable expression typically characterized by cardiac structural defects, most commonly atrial and ventricular septal defects, in association with upper limb deformity, particularly radial ray abnormalities. The genetic mechanism that simultaneously modifies cardiac and limb development is unknown. We have studied three unrelated kindreds affected by Holt-Oram Syndrome. In addition to variable intrafamily expression, the Holt-Oram Syndrome phenotype varies between families and ranges from severe cardiac structural and conduction abnormalities in the setting of mild to moderate radial ray deformity to absent or mild cardiac disease in association with moderate to severe upper limb malformation, including frank phocomelia. Despite the the gamut of phenotypes, the disease gene in all families analyzed maps to the same locus on the long arm of human chromosome 12 (maximum multipoint LOD score greater than 16). We hypothesize that interfamily phenotype variability derives from different mutations at the Holt-Oram Syndrome locus. Candidate genes currently under evaluation include transcription factors, cytoskeletal elements, cytokines, and extracellular matrix proteins. Genes for a retinoic acid receptor subunit and a HOX gene cluster both of which are known to modulate limb development and map to the vicinity of the Holt-Oram Syndrome locus have been excluded. Further efforts to identify the Holt-Oram Syndrome gene will help to elucidate molecular mechanisms that regulate cardiac and limb morphogenesis.

D 504 MOLECULAR ANALYSIS OF THE PRADER-WILLI / ANGELMAN CRITICAL REGION IDENTIFIES A CLUSTER OF IMPRINTED GENES. A.L. Beaudet, M. Nakao, B.A. Durtschi, A. Mutirangura, D.H. Ledbetter and J.S. Sutcliffe. Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct clinical disorders resulting from paternal and maternal deficiencies respectively in chromosome 15q11-q13. The data suggest the presence of oppositely imprinted genes in the region, and the gene encoding the small nuclear ribonucleoprotein-associated peptide N (SNRPN) has recently been identified as a candidate gene for PWS. We have completed a YAC contig spanning the entire PWS/AS critical region and developed a pulsed-field map from 100 kb centromeric to SNRPN across a Japanese deletion breakpoint (~200 kb telomeric) separating the PWS and AS critical regions to include an additional ~550 kb in the Angelman critical region.

A number of cDNAs representing at least six genes (PAR1-PAR6) in the PWS/AS critical region have been isolated, of which five have been positioned on the pulsed-field map. Partial sequence information is available for all of these cDNA clones. A gene located in the AS critical region was identified as a previously characterized gene encoding human papilloma virus E6-associated protein (E6-AP). RT-PCR analysis of deletion patient fibroblast and lymphoblast RNAs has been employed to assess the imprinting status of these genes. PAR2 and E6-AP which are located in the AS critical region are expressed from both maternal and paternal alleles. SNRPN, PAR5 and PAR1 were found to be expressed exclusively from the paternal chromosome. These three paternally-expressed genes define an imprinted domain spanning minimally 200 kb in the PWS critical region extending telomeric beyond the Japanese breakpoint. It will be possible to correlate the expression of each of these genes with the PWS phenotype in patients with paternal duplications or with small deletions localized to the SNRPN region. PAR5 and PAR1, which represent additional candidates for PWS, currently identify no functional homology in the sequence databases, but are under ongoing characterization.

D 505 CLONING cDNAs FROM 17q12-23 USING INTERSPECIFIC SOMATIC CELL HYBRIDS AND SUBTRACTIVE HYBRIDIZATION, Karen M. Cerosaletti, Michael H. Shapero, and R E. Keith Fournier, Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The region q12-21 on human chromosome 17 has recently been linked to early-onset familial breast cancer. To clone cDNAs from 17q12-21 expressed in breast tissue, we have utilized subtractive hybridization to generate region-enriched probes from rodent-human somatic cell hybrids containing fragments of Hsa 17. Two microcell hybrid cell lines were generated by transferring nearly isogenic fragments of Hsa 17 into mouse mammary epithelial cells. cDNA probes were synthesized from a microcell hybrid cell line containing a fragment of Hsa 17 which included the 17q12-23 region, encompassing the BRCA1 locus. Using subtractive hybridization, the cDNA probes were enriched for sequences from 17q12-23 with driver mRNA from a second microcell hybrid cell line which did not contain the 17q12-23 interval. The resulting subtracted cDNA probes were used to screen a human pre-menopausal breast cDNA library, yielding 471 primary positive pools. The inserts of the primary positive pools were amplified by the polymerase chain reaction and screened with a second 17q12-23-enriched cDNA probe to isolate 56 secondary positive pools. Individual plaques reacting with the 17q12-23-enriched probes were then identified in a tertiary screen using a subtracted cDNA probe. cDNAs were mapped by Southern blot analysis to identify genes encoded in the region of non-overlap between the parental microcell hybrid cell lines. Five cDNAs mapped to the non-overlap region 17q12-23. Three cDNAs represent new genes in the database, while one has homology to poly A binding protein and a ribosomal protein, L35.

D 506 CORRECTION OF THE DEFECT IN ATAXIA-TELANGIECTASIA COMPLEMENTATION GROUP E CELLS

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Queensland Institute of Medical Research, Bancroft Centre, 300 Herston Road, Brisbane 4029, Australia.

Ataxia-telangiectasia is characterized by immunodeficiency, developmental abnormalities, neurodegenerative changes, chromosomal instability, hypersensitivity to ionising radiation and predisposition to cancer. Gatti et al (1988) localised the gene for A-T complementation group A to chromosome 11q22-23. A putative A-T gene (group D) has been isolated by transfection with a human cosmid clone library (Kapp et al, 1992). Positional cloning is also continuing in order to identify an A-T gene. In order to identify the A-T complementation group E gene we have transected lymphoblastoid cells with a pREP4 cDNA library obtained from M. Buchwald (Toronto). Selection was carried out in hygromycin (150ug/ml) followed by radiation exposure. The surviving cells were tested for radiation resistance and cells were used to recover episomal DNA by alkaline lysis extraction followed by electroporation into bacteria. 50 clones were examined by gel electrophoresis for cDNA inserts. Surprisingly a few predominant clones were identified containing either 2.2, 2.5 or 4.5 kb inserts. DNA from these clones was re-transfected into A-T cells to determine whether normal radiation resistance had been fully restored. We are at present in the process of characterising transfectants using 6 different criteria for radioresistance.

D 507 **Fine mapping of five additional short tandem repeats (STRs) within the Prader Willi/Angelman syndrome critical region on chromosome 15q11.2-12,**
Susan L. Christian[1], Apiwat Mutirangura[3], Wendy P. Robinson[4], Mitsuyoshi Nakao[1,2], Arthur L. Beaudet[1,2] and David H. Ledbetter[1], [1]Institute for Molecular Genetics, [2]Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, [3]Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand, [4]Institut für Medizinische Genetik der Universitat Zürich, Zürich, Switzerland.

The Prader-Willi (PWS) and Angelman (AS) syndromes are mental retardation disorders with distinct phenotypes associated with a lack of paternal (PWS) or maternal (AS) inheritance of chromosome 15q11.2-12 through either deletion or uniparental disomy. A ~3.5 MB YAC contig of this region has recently been developed (Mutirangura et al., Genomics, in press) allowing the development of new STRs within this region and fine mapping of previously identified STRs. Alu-CA PCR was used to develop one STR from YAC A124A3 containing the IR39 (D15S18) locus while vectorette PCR identified another STR from a cosmid mapping to the proximal end of YAC 254B5. A third STR was discovered within a cosmid distal to the SNRPN gene (a candidate gene for PWS). The YAC contig also allowed precise mapping of two previously identified STRs. D15S122 mapped within YACs 230H12, 132D4, and B230E3 placing this marker between TD3-21 (D15S10) and LS6-1 (D15S113) while D15S128 mapped near the SNRPN locus contained within YACs 457B4 and 11H11.

Multiplex PCR utilizing these new STRs has allowed the identification of a small deletion near the SNRPN gene in a patient with Prader-Willi syndrome which could not be detected using the other STRs within this region. Additional patients can now be screened for microdeletions near the SNRPN gene to facilitate diagnosis of Prader Willi syndrome. Additionally, these new STRs provide additional polymorphic markers needed to confirm the parental origin of uniparental disomy in both Prader Willi and Angelman syndromes.

D 508 IDENTIFICATION OF A COPPER-BINDING ATPase HOMOLOGOUS TO THE MENKES GENE: A CANDIDATE FOR THE WILSON DISEASE GENE, Diane Wilson Cox, Peter C. Bull, Gordon R. Thomas, Johanna M. Rommens, John R. Forbes,
Research Institute, The Hospital for Sick Children, Toronto, ON M5G 1X8.
Copper is an essential trace metal for both prokaryotes and eukaryotes, as an essential component of many enzymes and structural proteins. Dietary intake of copper generally far exceeds the trace amounts required, and effective means for elimination of the excess have evolvrd. Although several copper proteins involved in transport have been identified, the mechanism of copper efflux from tissues has remained an enigma. Two disorders of copper transport have been identified; Menkes disease, an X-linked disease in which copper is not transferred from intestinal cells, and Wilson disease.

Wilson disease (hepatolenticular degeneration) is an autosomal recessive disorder of copper transport, resulting in accumulation of copper in the liver, brain, *kidney, and cornea*. We have previously isolated new markers in the region of the gene on chromosome 13q14.3. We have used them to construct a long range restriction map and to obtain 19 YACs in the region. Using the copper-binding motif of the ATPase defective in Menkes disease, we identified a homologous region on three overlapping YACs, and on cosmids from a chromosome 13 library. Cosmids were used to isolate cDNA clones by a direct PCR-based cDNA selection strategy. The sequence of the gene identified shows considerable homology with the Menkes ATPase throughout all its functional domains, including at least 6 copper-binding domains, transduction, phosphorylation, and ATP-binding domains. There are no other similar domains in the region, or on chromosome 13.

Chromosomal localization supports this gene as a candidate gene for Wilson disease(WND). The gene has previously been mapped between markers D13S59 and D13S31. We have shown allelic association of WND with D13S31. We have developed 3 new CA repeat markers within the WND region (D13S314, D14S315, D14S316). Two of these, in addition to the previously reported marker D13S133, lie within a 300 kb region and show strong allelic association with WND. In addition, one of these markers defines the proximal boundary of the WND region in a recombinant individual. The putative copper-binding ATPase lies within this 300 kb region.

The proposed candidate gene is expressed in liver, where there is scarcely any expression of the MNK gene. This expression pattern is compatible with the defective transport of copper from the liver in patients with Wilson disease.

D 509 ASCERTAINMENT OF 22q11 MICRODELETIONS IN PATIENTS WITH CONGENITAL HEART DISEASE, Elizabeth Goldmuntz, Deborah Driscoll, Beverly S. Emanuel, Division of Molecular Biology and Human Genetics, The Children's Hospital of Philadelphia, Philadelphia, PA, 19104

Recent work has demonstrated that patients with DiGeorge syndrome (DGS) and Velo-cardio-facial syndrome (VCFS) have overlapping microdeletions of 22q11. Both syndromes are characterized in part by congenital heart disease. DGS is most frequently associated with truncus arteriosus (TA), interrupted aortic arch (IAA) or tetralogy of Fallot (TOF), while VCFS is commonly associated with TOF or ventricular septal defects (VSD). We previously demonstrated that 5 of 17 non-syndromic patients with TA, IAA or TOF have 22q11 microdeletions. We have now evaluated 49 non-syndromic patients with TA, IAA or TOF. We have also begun screening patients with other conotruncal defects including transposition of the great arteries (TGA) and double outlet right ventricle (DORV), as well as those with VSD. Initially, copy number at a test locus was determined by Southern blot dosage analysis. We have recently applied the technique of fluorescence *in situ* hybridization (FISH) to detect deletions. Biotinylated cosmid probes that map to the deleted region of 22q11 and a control probe that maps to the distal long arm of chromosome 22 are hybridized with metaphase chromosomes. Thus far, 4 of 9 patients with TA, 1 of 9 with IAA, 4 of 31 with TOF have 22q11 deletions. To date, patients with TGA (8) or DORV (2) have not demonstrated 22q11 deletions, although the numbers evaluated are small. However, 2 of 10 patients with VSD, not diagnosed with VCFS upon enrollment into the study, have 22q11 deletions. These studies begin to identify a subgroup of congenital heart disease (CHD) patients with 22q11 microdeletions. Although CHD is usually diagnosed in infancy, the apparently non-syndromic, cardiac patient with a 22q11 deletion may be at risk for the associated features of VCFS that do not manifest themselves until school age. Early recognition of this risk, however, can lead to early diagnosis of the associated features and improved treatment.

D 510 GENETIC ANALYSIS OF CANINE X-LINKED SEVERE COMBINED IMMUNODEFICIENCY, Paula S. Henthorn, Suzanne M. Deschênes, Amalia S. Dutra, Geoffrey M. Jackson, Richard L. Somberg, Peter J. Felsburg and Jennifer M. Puck, Sections of Medical Genetics and Immunology, Sch. of Vet. Med., and Depts of Pediatrics and Genetics, Sch. of Med., University of Pennsylvania, Philadelphia, PA 19104

The human and canine syndromes of X-linked severe combined immunodeficiency (X-linked SCID) are characterized by similar clinical and immunological manifestations. The human X-linked SCID gene locus (SCIDX1) is located in Xq13 and has been identified as the gene encoding the gamma chain of the interleukin-2 receptor (IL2RG). For genetic analysis of canine X-linked SCID, polymorphic markers were developed from genes located in human proximal Xq near SCIDX1. Canine genomic phage clones hybridizing to a poly(dA-dC)•poly(dG-dT) probe and to human choroideremia (CHM) or to phosphoglycerate kinase (PGK1) sequences were found to contain $(TG)_n$ polymorphisms informative in the canine X-linked SCID colony. These plus a polymorphic $(CAG)_n$ sequence in exon 1 of the canine androgen receptor gene (AR) were used to genotype dogs informative for X-linked SCID. No recombinations between SCIDX1, AR, PGK1, or CHM were observed in the dog; in contrast, canine SCIDX1 and the canine dystrophin locus (CXMD) were found to be 22 cM apart. Fluorescence *in situ* hybridization localized PGK1 and CHM to proximal Xq in the dog, in the same chromosomal location occupied by the human genes. Somatic cell hybrid analysis and methylation differences at AR demonstrated that female dogs carrying X-linked SCID have the same lymphocyte-limited skewed X-chromosome inactivation patterns as human X-linked SCID carriers. Preliminary cloning and sequence analysis of the canine IL2RG gene and of cDNAs from normal and affected dogs indicates that X-linked SCID in these animals is caused by a four base pair deletion in the first exon of the IL2RG gene. These findings provide direct genetic evidence that mutations in the same gene cause canine and human X-linked SCID and establish dog X-linked SCID as an excellent model for the development of gene transfer protocols for the treatment of immunodeficiencies.

D 511 FAMILIAL BREAST/OVARIAN CANCER: PROGRESS TOWARDS ISOLATION OF A PREDISPOSING GENE, Karen Jones, Donald Black, Hans Nicolai, Beatrice Griffiths, Marisa Bonjardim, Melissa Brown, Michael North, Leo Schwalkwyk, Hans Lehrach, Bernhardt Korn, Annemarie Poustka and Ellen Solomon, Somatic Cell Genetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX

Both breast and ovarian cancer arise due to DNA alterations which occur specifically in the somatic tissues or in the germline. Families have been identified in which members inherit a germline alteration which increases susceptibility to breast or ovarian cancer. These families have enabled the mapping of an important predisposing gene, *BRCA1* to a small region on chromosome 17q21.

The isolation of *BRCA1* requires a positional cloning strategy: the correlation of the genetic map of the region into a physical map and saturation of the region with YAC and cosmid clones . Mini cDNA libraries enriched for the region of interest are being generated using a method of hybridisation of cDNA libraries to biotinylated cosmid DNA from the region (Korn *et al*, 1992). Together with the method of exon-trapping from these cosmids (Buckler *et al*, 1991), new cDNAs are being isolated and their involvement in breast and ovarian cancer is under analysis.

Identification of such a gene will enable prevention, early diagnosis and possibly novel treatments in such families and in women generally if the gene is also important in the development of purely somatic disease.

D 512 HOMOZYGOUS DELETION AT CHROMOSOME 2q33 IN HUMAN SMALL-CELL LUNG CARCINOMA IDENTIFIED BY AP-PCR GENOMIC FINGERPRINTING,Takashi Kohno (1), Kazuhiro Morishita (1), Hirokuni Takano (1), David N. Shapiro (2) and Jun Yokota (1), National Cancer Center Res. Inst, Japan (1) St Jude Children's Research Hospital, U. S. A. (2)

We have searched for novel genetic alterations in human cancer cell lines by using the Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), which is a PCR-based genomic fingerprinting. A homozygous deletion was detected in a small-cell lung carcinoma (SCLC) cell line. Since homozygous deletion is a critical genetic alteration for the inactivation of tumor suppressor gene, we defined the locus of homozygous deletion. Fluorescence *in situ* hybridization analysis revealed that the deletion was localized at chromosome 2q33. The size of the homozygous deletion in the SCLC cell line was more than 20 kilobase pairs. Seven loci mapped to 2q32-qter were all retained in this cell line, suggesting the presence of submicroscopic interstitial deletion in this cell line. Considerably high incidence of allelic loss on chromosome 2q was detected in SCLC, non-SCLC, colorectal carcinoma and neuroblastoma by restriction fragment length polymorphism analysis. These results suggest that the locus for a novel tumor suppressor gene, which is involved in the development of several human cancers, was mapped to 2q33.

D 513 CANDIDATE GENES FOR MULTIPLE ENDOCRINE NEOPLASIA TYPE I, Jacob Lagercrantz, Günther Weber, Eitan Friedman, Sean Grimmond, Nicholas Hayward, Magnus Nordenskjöld, Catharina Larsson, Dept. of Clinical Genetics, Karolinska Institute, Stockholm, Sweden

Multiple Endocrine Neoplasia type 1 is characterized by neoplasia of the parathyroids, the pancreas and the pituitary. Tumorigenesis involves unmasking of a recessive mutation at the MEN1 locus which has been mapped to the centromeric part of the chromosomal region 11q. We have isolated new cosmid markers from radiation reduced somatic cell hybrids that contained the MEN1 region as the only human component. RFLPs of the newly isolated cosmid clones were used to detect meiotic cross-overs in MEN1 families, and to study deletions (loss of heterozygosity) in familial and sporadic MEN1 related tumors. Combined with physical mapping (PFGE) the region for the MEN1 gene was narrowed to less than 1MB. With cosmids from this region cDNA clones from a human fetal brainlibrary were isolated and used in expression and tumor deletion studies. One of the cDNA clones analyzed were found to be ubiquitously expressed but had completely lost expression in some MEN1 related tumors. This was done both using Northernblot and in situ hybridization techniques. We therefore conclude that this or a closely located gene is responsible for MEN1. This candidate gene are now subjected to extensive investigations including mutation analysis, determination of genomic structure and further expression studies.

D 514 IDENTIFICATION OF A NOVEL GENE PRODUCING PHENOTYPIC COMPLEMENTATION OF FANCONI ANEMIA FIBROBLASTS, Robb E. Moses, Michael A. Whitney, Markus Grompe, Louise S. Merkens, Petra M. Jakobs, James A. Hejna and Thomas V. Tittle, Oregon Health Sciences University, Department of Molecular and Medical Genetics, Portland, Oregon 97201

Cells from patients with Fanconi anemia (FA) display chromosomal instability and increased sensitivity to mitomycin C (MMC) and Diepoxybutane (DEB) relative to normal cells. Four complementation groups have been identified indicating several genes acting in a pathway of DNA damage repair. These genes may be required for normal forearm development and marrow cell growth as FA is associated with defects in these processes. We are attempting to identify FA genes other than the FA-C group (Strathdee *et al.* Nature 356:763 [1992]). We have constructed a cDNA expression library in a vector designed for chromosomal integration with a G418 selectable marker. Approximately 1 x 10^6 independent cDNA clones with an average insert size of 1.5 kb were isolated following unidirectional ligation behind a cytomegalovirus (CMV) promoter. Five MMC resistant cell lines were selected from 6 x 10^6 G418-resistant transfectants obtained from 65 individual transfections of the FA-A fibroblast cell line GM6914. The isolated cell lines also showed normal resistance to DEB and normal chromosome stability. The cDNA from the MMC-resistant cells was recovered by PCR from flanking sequences in the vector. One recovered cDNA had an apparent size of 1.4 kb corresponding to a 1.2 kb cDNA insert. It was detected in two of the five corrected cell lines. Retransfection of this cDNA (PCR product) into GM6914 again complemented the MMC repair defect. DNA sequencing showed an open reading frame of approximately 1 kb. The nucleotide sequence does not match any sequence in GenBank. The genomic structure for the cDNA is relatively simple, with two short introns. Using cell lines from the OHSU Fanconi Anemia Cell Repository, mutation analysis did not identify any mismatches with cDNA from GM6914 or from eight other FA patients. Interestingly, the cDNA was localized to chromosome 11q in the region linked to ataxia telangiectasia.

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D 515 THE 3;21 TRANSLOCATION IN MYELODYSPLASIA RESULTS IN TWO FUSION TRANSCRIPTS BETWEEN THE *AML1* GENE AT 21q22 AND TWO GENES LOCATED AT BAND 3q26, Giuseppina Nucifora, Catherine R. Begy, Hirofumi Kobayashi, Evan Parganas, James N. Ihle and Janet D. Rowley, Dept of Medicine, University of Chicago, Chicago, IL 60637 and Dept of Biochemistry, St. Jude Children's Hospital, Memphis, TN 38105

A recurring translocation between chromosomes 3 and 21, t(3;21)(q26;q22), has been detected in patients with chronic myelogenous leukemia in blast crisis (CML-BC) or with therapy-related myelodysplastic syndrome or acute myeloid leukemia (t-MDS/t-AML). In these patients, the breakpoint on chromosome 21 is at band 21q22, which is also involved in the t(8;21)(q22;q22) associated with acute myeloid leukemia subtype M2 (AML-M2). In the t(8;21), the *AML1* gene is the site of the breakpoint on chromosome 21. *AML1* is homologous to the DNA binding α subunit of the T cell enhancer CBF. In the t(8;21), *AML1* fuses with *ETO* to form a chimeric gene. The *AML1/ETO* fusion mRNA has been consistently identified in several AML patients with a t(8;21). We have isolated a fusion cDNA clone from a t(3;21) t-MDS patient's library that contains sequences from *AML1* and *EAP* which we have localized to band 3q26. EAP has been previously characterized as a highly expressed small nuclear protein of 128 residues (EBER 1) associated with Epstein-Barr virus small RNA. The fusion clone contains the 5' DNA binding part of *AML1* that is fused to *ETO* in the t(8;21) and at least one other exon. The t(3;21) replaces the carboxyl end of *AML1* with the last 96 codons of *EAP*. The fusion does not maintain the correct reading frame of *EAP*, and may not lead to a functional chimeric protein. We have identified a second fusion gene from the same patient's library. This clone contains the same region of *AML1* found in the *AML1/EAP* mRNA but it is fused in frame to a new gene, *MDS1*, which is evolutionarily conserved with no homology to sequences deposited in GenBank. Both fusion mRNAs have been detected by reverse transcription-polymerase chain reaction in peripheral blood cells of this patient. Combined results of pulsed field gel electrophoresis (PFGE) and in situ hybridization (FISH) mapping have shown that the two genes are located at 3q26 near *EVI1*, with *EAP* the most telomeric and *EVI1* the most centromeric of the three genes. Because the other chromosomes 3 and 21 appear cytogenetically normal, these results suggest that the two fusion mRNAs could be obtained by differential splicing of a common primary nuclear transcript that includes *AML1* and both the *EAP* and *MDS1* genes.

D 516 LINKAGE STUDIES IN CYSTINURIA, E. Pras[1], N. Arber[2], J. Shapiro[2], I. Aksentijevich[1], G. Katz[4], D. Harell[2], U. Liberman[2], M. Pras[3], D. L. Kastner[1],[1]ARB, NIAMS, NIH, Bethesda MD; [2]Beilinson Medical Center, Petah-Tiqva,[3]Sheba Medical Center, Tel-Hashomer, [4]Hadasa Medical Center, Jerusalem, Israel.

Cystinuria is an autosomal recessive disease that involves the transport of cystine and the dibasic amino acids arginine, lysine and ornithine. It is manifested by cystine kidney stones, which can lead eventually to renal failure. The disease is responsible for 1-2% of all kidney stones and 6-8% of kidney stones in children. The metabolic defect has been localized to the brush border of the proximal renal tubule, and to the intestinal epithelium, but its exact nature and chromosomal location remain unknown. As a first step toward identifying the specific genetic defect causing the disease, we undertook a genome wide search for linkage in cystinuria families. We obtained DNA samples from 9 such families, a total of 52 individuals, 22 of whom are affected. Using polymorphic DNA markers we screened over 50 loci *on 16 different* chromosomes and found no evidence of linkage. Recently a human kidney cDNA involved in cystine, dibasic and neutral amino acid transport has been mapped to chromosome 2. Obviously a mutation in such a gene would be a very likely cause of cystinuria. From a total of 14 chromosome 2 markers that we have checked, the locus D2S119 gave a maximal lodescore of 2.43 at θ=0.08. Currently we are checking more cystinuria families as well as additional markers to try to establish definite linkage.

D 517 CHARACTERIZATION AND CLONING OF THE CRITICAL REGION FOR THE MICROPHTHALMIA WITH LINEAR SKIN DEFECTS SYNDROME (MLS). L. Schaefer[1], M. C. Wapenaar[1], M. T. Bassi[1], G. B. Ferrero[1], A. Grillo[1], E. J. Roth[2], A. Ballabio[1], and H. Y. Zoghbi[1,2], [1]Institute for Molecular Genetics and [2]Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030, USA

We have collected cell lines from 17 female patients with deletions and/or translocations resulting in the loss of the terminal portion of Xp22.3-Xp22.2. These patients have complex phenotypes which include one or more of the following features: microphthalmia, linear skin defects, agenesis of the corpus callosum, retinal abnormalities, seizures, mental retardation, and skeletal abnormalities. A combination of these findings have been observed in three X-linked male-lethal disorders, Aicardi syndrome, Goltz syndrome, and microphthalmia with linear skin defects syndrome (MLS). The overlap in the phenotype suggests that the same gene or genes are involved in all three disorders. Because these disorders are male-lethal, we hypothesized that the candidate region for gene(s) causing the phenotype in our patients (which we refer to as MLS) is proximal to the deletion breakpoints in males with Xpter-Xp22 nullisomy and distal to the breakpoints of female patients with the MLS phenotype and Xpter-Xp22 monosomy. To map the MLS critical region, somatic cell hybrids from eleven of the MLS patient cell lines were generated to separate the derivative X chromosome from the normal one. These hybrid cell lines as well as cell lines from male patients with Xpter-Xp22 deletions constituted our mapping panel and allowed us to define the distal and proximal boundaries of the MLS critical region after analysis of the mapping panel with 28 existing and newly developed markers from Xp22. Using sequence-tagged sites at markers mapping to Xp22, a 1.5 megabase yeast artificial chromosome (YAC) contig has been developed which spans the critical region. Long-range restriction analysis of the YACs using nine rare-cutting enzymes allowed us to estimate the size of the MLS candidate region to be 450-550 kb. Cosmid clones have been identified within the critical regon using the Livermore X-chromosome cosmid library and a cosmid library constructed from one of the YACs in the critical region. The cosmid and YAC clones are currently being used in several strategies aimed at isolating expressed sequences from this region, including cDNA selection, exon-trapping, and cross-species conservation studies. To date, one terminal exon and four evolutionarily conserved sequences have been isolated within the critical region; these sequences are being used to screen cDNA libraries.

D 518 MAPPING OF HUMAN ZINC-FINGER cDNAs TO REGIONS ASSOCIATED WITH GENETIC/MALIGNANT DISEASES, Niels Tommerup, Esper Boel and Henrik Vissing, J.F.Kennedy Institute, DK-2600 Glostrup, Denmark; Biosciences Division, Novo Nordisk A/S, Bagsvaerd, Denmark.

Transcriptional regulatory proteins containing tandemly repeated zinc finger domains (ZNFs) are thought to be implicated in both normal and abnormal cellular proliferation and differentiation. The genome may contain several hundreds of these candidate genes for developmental and malignant disorders. We have isolated 28 ZNF cDNAs from an insulinoma cDNA library, the first 13 of which have been given ZNF numbers (ZNF131-143). The first 21 of these cDNAs were mapped to the following chromosome bands: 1p36.1;1p21;2q35;3q21;4p16.3;5p11; 5q33;7q11.21;7q21.3;11p15.4;12p13;12q24.33;19p13.1;19q13.2;19q34 ;20p11.2, with clustering at 19q34 and 20p11.2. It was necessary to combine fluorescence in situ hybridization (FISH) with somatic cell hybridization to map some of the cDNA clones due to cross-hybridizing signals. Several of the cDNAs mapped to chromosomal regions implicated in specific genetic/malignant disorders: pHZ-67 maps to 1p36.1, a region commonly deleted in neuroblastoma. pHZ-52 maps to 3q21, within a region associated with the blepharophimosis, ptosis, epicanthus inversus (BPES) syndrome. ZNF141 maps within the 2.2 Mb smallest region of deletion overlap at 4p16.3 associated with the Wolf-Hirschhorn (4p-) deletion syndrome. pHZ-97 maps to 5q33, a region deleted in myelodysplastic syndrome. ZNF138 mapping at 7q11.21 may be of interest for Williams syndrome. ZNF143 maps to 11p15.4, a region associated with Beckwith-Wiedemann syndrome and loss of heterozygosity in Wilms tumor. pHZ-58 mapping at 12p13 is of interest in testicular cancer where iso(12p) is a frequent early finding. Two cDNAs mapped at 20p11-p12, close to the region deleted in Alagille syndrome. The elucidation of the possible role of these ZNFs in the various disorders will include complete sequencing, expression studies, FISH analysis of disease-specific chromosomal rearrangements and in some cases search for DNA binding sequences.

D 519 A ZINC-FINGER GENE ZNF141 MAPPING AT 4p16.3 IS A CANDIDATE GENE FOR THE WOLF-HIRSCHHORN (4p-) SYNDROME. Henrik Vissing[1], Esper Boel[1], Sarah Baxendale[2], Gillian Bates[2], Hans Lehrach[2] and Niels Tommerup[3]. [1]Bioscience, Novo Nordisk, DK-2880, Bagsvaerd, Denmark, [2]Department of Genome Analysis, Imperial Research Cancer Foundation, London, UK, [3]The John F. Kennedy Institute, Glostrup, Denmark.

Wolf-Hirschhorn syndrome (WHS) is a mid-line fusion syndrome caused by deletion of the short arm of chromosome 4 (4p-) and is characterized by delayed growth and abnormal development of a variety of organs, including brain, heart and kidneys. The smallest region of overlap suggest that the gene(s) associated with the WHS phenotype is located within a 2.2 Mb subtelomeric region of 4p, flanked by the anonymous loci D4S43 and D4S142. We have isolated several full-length cDNAs containing zinc finger motifs, and have fine mapped these directly to high resolution R-banded mitotic chromosome by fluorescence in situ hybridisation (FISH). One cDNA, ZNF141, mapped within the telomeric region of the short arm of chromosome 4 (4p16.3). In order to asses its relationship to WHS, we have mapped it by FISH with respect to selected translocation breakpoints on 4p, and to an ordered set of cosmids from the distal 4p region. ZNF141 mapped to the distal end of the 2.2 Mb WHS region, 300 kb from the 4p telomere on cosmid CD1 defining the anonymous locus D4S90. ZNF141 belongs to the C_2-H_2 class of zinc finger proteins and was isolated from a human insulinoma cDNA library. The nucleotide sequence identified an open reading frame of 1,422 bp with ten finger motifs that all conform to the general pattern $\mathbf{CXXCX_3FX_8HXXXH}$. In addition, a segment present within the N-terminus was identified as a KRAB domain. ZNF141 is thus a putative transcriptional regulator. Northern blot analysis of ZNF141 with mRNA from adult tissue did not reveal any specific hybridisation, however, a competitive RT-PCR analysis using the ubiquitously expressed cyclophilin as competitor, demonstrated the presence of ZNF141 message in all tissue analysed, indicating a ubiquitously low expression. ZNF141 fulfils the initial criteria that must be met for a candidate gene for WHS. It maps within the critical chromosome region; it contains an open reading frame, and it is expressed. It is the first ZNF gene that has been mapped to chromosome 4, and in addition it is the most telomeric gene that has been identified on 4p so far. The DNA binding domain of ZNF141 has been expressed as a GST fusion protein, which is being use to identify its DNA target. The identification of putative target DNA sequences and the possible role of ZNF141 in transcriptional regulation may shed light on its involvement in the pathobiology of WHS.

D 520 GENETIC AND PHYSICAL MAPPING OF THE REGION OF HUMAN CHROMOSOME 2q CONTAINING IL-8R GENES AND THE MACROPHAGE RESISTANCE GENE NRAMP.

Jacqueline K. White, Marie-Anne Shaw, Susan Searle, C. Howard Barton and Jenefer M. Blackwell, University of Cambridge Clinical School, Department of Medicine, Addenbrooke's Hospital, Cambridge, CB2 2QQ, U.K.

Resistance to the macrophage pathogens *Leishmania donovani*, *Salmonella typhimurium* and *Mycobacterium bovis* is controlled by a single gene (*Lsh/Ity/Bcg*) located on the proximal region of mouse chromosome 1. Recently Vidal and coworkers (*Cell* **73**:469-485, 1993) employed a positional cloning strategy to identify a candidate for *Lsh/Ity/Bcg*, designated *Nramp* (Natural resistance associated macrophage protein). To determine the potential role of this gene in human disease, genetic and physical mapping of the homologous region human chromosome 2q has been undertaken. Linkage analysis between 2q markers and a putative disease susceptibility locus in multicase families of leprosy or tuberculosis has so far failed to reveal a role for a major single gene influence in this region. A larger dataset of affected relative pairs should reveal whether a gene in this region contributes to multifactorial inheritance of disease susceptibility. Long range physical mapping studies of the region encoding NRAMP and the tightly linked markers IL-8R (3 genes: type I, type II and the pseudogene) and VIL1 have been undertaken using yeast artificial chromosome (YAC) clones isolated from the CEPH, ICI and ICRF human YAC libraries. Collinearity of the YACs isolated was first examined by FISH, and all were screened for the presence of NRAMP sequence and the marker genes. One clone, in particular, has provided the basis to development of a high resolution physical map of the region. This YAC has also been subcloned into EMBL3 and screened for full-length genomic clones for NRAMP. Detailed sequence analysis of these genomic clones, in conjunction with cDNA sequence data, is being undertaken to facilitate development of rapid screening procedures for mutations identified in the cDNA sequence of human families with visceral leishmaniasis.

D 521 EXCLUSION OF LINKAGE OF REFSUM'S DISEASE TO MAJOR LOCI FOR RETINITIS PIGMENTOSA,

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Refsum's disease (heredopathia atactica polyneuritiformis) is a autosomal recessive clinical syndrome distinguished by retinitis pigmentosa, chronic polyneuritis and cerebellar ataxia presenting between 3 and 60 years of age. The biochemical defect is probably a mutation in the unisolated peroxisomal enzyme phytanic acid α-oxidase which degrades the α-methylated fatty acid- phytanic acid (derived from chlorophyll via phytol) to a form suitable for β-oxidation. This study set out to localise the gene for Refsum's disease by examining its linkage to loci known to be associated with retinitis pigmentosa (RP). Four families comprising 34 members with 9 affected individuals spanning 3 generations and including one consanguineous family were recruited. Linkage to the known RP loci on chromosome 3q21, 8p11-22, 6p and 7q31-35 was sought by using polymorphic CA repeat haplotype mapping and the CA repeat markers: rhodopsin [3q21-24], ssT [3q24], D3S196 [3q27-28], LPL [8p22], ankyrin [8p11.1], D8S87 [8p15], D6S109 [6p24-21.1], FTHP1 [6p21.3-12], F13A1 [6p24.2-23], TNF-β [6p21.3] and CFTR [7q31.3]. Haplotypes were determined by polymerase chain reacton amplification and run on polyacrylamide-urea gels. The results were analysed using the program 'Linkage' (HGMP Resource Centre). The analysis in these families excluded linkage to these RP loci: LOD scores of > -2.0 were found at $\theta = 0.005$-0.01 (3q), $\theta = 0.005$-0.01 (8p), $\theta = 0.0$-0.001 (6p) and $\theta = 0.1$-0.05 (7q) in those markers that were informative and assuming that the disease was fully penetrant as determined by plasma phytanic acid levels. This suggests that the pathogenesis of retinitis pigmentosa in Refsum's disease is different from that described in other forms of this ocular syndrome.

D 522 CATALOGUING HUMAN GENES BY SINGLE PASS AND FULL LENGTH SEQUENCING AND PHYSICAL AND GENETIC MAPPING OF BRAIN cDNAS, Wilcox, A.S., Stevens, T.J., Berry, R., Rubano, T., Walter, N., Hopkins, J.A., Glod, J., Orpana, A.K. and Sikela, J.M. Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, Colorado 80262

We have performed single pass sequencing of more than 3,000 human brain cDNAs, most of which seem to represent new human genes. Single pass sequencing is used to identify non-redundant cDNAs that are then used for full-length sequencing and physical/genetic mapping. We illustrate how these approaches can be efficiently and systematically integrated to maximize the information obtained from high throughput sequence-based cDNA analyses.

For physical mapping, primers designed from 3'untranslated sequences are used for PCR analysis of CEPH megabase YAC pools. A single YAC corresponding to the cDNA can be identified using only 52 PCR reactions. Emergence of megabase YAC contigs makes it increasingly likely that assignment of a cDNA to a YAC will also yield the specific chromosomal location of the gene. cDNA-positive YACs which are not in contigs are used for FISH analysis to obtain both regional mapping data and to check for YAC chimerism. We have also identified a subset of human cDNAs containing polymorphic microsatellite sequences and show how they can be converted to highly informative (PIC >0.7) markers that permit integration of the human physical, expression and genetic maps.

For full length cDNA sequencing we have explored using the presence of a 5' located *NcoI* site as a predictor of cDNAs that contain consensus translational start sites and thus are likely to contain complete protein coding regions. We have also improved the *ExoIII/ExoVII* unidirectional deletion strategy to permit high throughput automated sequencing of complete cDNA inserts using existing ABI373A sequencing instrumentation.

Gene Expression

D 600 A GENE FOUND IN THE DIGEORGE REGION OF CHROMOSOME 22Q11 IS EXPRESSED DURING CRITICAL PERIODS OF PHARYNGEAL ARCH DEVELOPMENT IN THE MOUSE EMBRYO, H. Scott Baldwin, Hong Min Shen, Craig S. Mickanin, Elizabeth Goldmuntz, Marcia Budarf, Deborah Driscoll, Beverly S. Emanuel, Clayton A. Buck, Division of Pediatric Cardiology, The Children's Hospital of Philadelphia, Philadelphia, PA., 19104

A proposed explanation for DiGeorge Syndrome (DGS) is through a perturbation of neural crest development which results in a characteristic pattern of malformations involving craniofacial, thymic, and conotruncal cardiac defects. The exact molecular mechanisms of DGS are poorly understood. Recently, a consistent hemizygous microdeletion of human chromosome 22q11 has been associated with DGS defining a DiGeorge Chromosome Region (DGCR). In order to begin delineating the candidate genes involved in DGS, we screened a mouse heart cDNA library with a partial human cDNA encoded by the distal region of the DGCR and obtained a unique 1.6 Kb cDNA that was 82% homologous at the nucleotide level with its human counterpart. Hybridization of a human somatic cell mapping panel with this murine cDNA confirmed a human homologue to this gene on Chromosome 22 which was deleted in DGS hybrids. Northern blot analysis of mRNA demonstrated a 4.2 Kb message in the head, heart and pharyngeal arch region of the 11.5 day mouse embryo. Initial whole mount *in situ* hybridization experiments revealed an accentuation of message in the cranial neural crest of the 8.5 day mouse embryo that persisted in the head, pharyngeal arches, as well as limb bud of the 9.5 day embryo. These data suggest that a unique gene found within the DGCR is conserved in the mouse and is expressed during critical periods of craniofacial and cardiac morphogenesis. No homologous genetic sequence has been found in the current Gene Bank. Several other candidate genes have been identified and are currently being characterized. Alterations in the transcription, translation, and expression of such "candidate genes" may provide insights into the pathogenesis of both DGS.

D 601 MOLECULAR GENETIC ANALYSIS OF BETA-GLOBIN EXTINCTION, E.S. DIEKEN and R.E. Keith Fournier, Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Extinction is the repression of tissue-specific gene expression that generally occurs in somatic cell hybrids formed by fusing different cell types. This repression is dramatic (>1000-fold) and is accompanied by changes in chromatin structure at tissue-specific loci. Resolving the genetic basis of extinction should provide valuable insights into the regulatory controls of developmental and tissue-specific gene expression. Recently, a region upstream of the human β-globin locus, referred to as the locus control region (LCR), has been implicated in regulating the transcription and chromatin structure of the entire β-globin domain. The human β-globin LCR is operationally defined by five DNase I hypersensitive sites located 6-20 kilobases 5' of ε-globin. Deletion of this region in certain thalassemias results in complete inactivation of the erythroid-specific, DNase I-sensitive chromatin domain and furthermore, a shift from early to late S-phase replication timing of the locus. It has been proposed that the LCR contains a novel regulatory element responsible for activation of the globin locus in erythroid cells. To determine whether the LCR is involved in β-globin extinction, mouse erythroleukemia cells (MEL) expressing a β-globin-β-galactosidase reporter transgene with or without the β-globin LCR were fused with a pre-B cell line, 1881. Transgene extinction of MEL-transfectant x 1881 hybrids was quantitated by measuring the inducible β-galactosidase activity using the methylumbelliferyl-β-D-galactoside (MUG) assay and by calculating the fold-reduction in mRNA levels using Northern blot analysis. Hybrids containing the -LCR transgene show a 10-fold reduction in β-galactosidase expression level whereas hybrids containing the +LCR transgene show a 500-fold reduction. These results indicate that extinction of the β-globin gene in MEL x 1881 hybrids is mediated through the LCR.

D 602 RT-PCR ANALYSIS OF THE t(2;13) TRANSLOCATION OF ALVEOLAR RHABDOMYOSARCOMA, J.R. Downing, D.Head, M.G. Hulshof, D.M. Parham, and D.N. Shapiro, Departments of Pathology, Tumor Cell Biology, and Experimental Oncology, St. Jude Children's Research Hospital, Memphis, TN 38105

Rhabdomyosarcoma, the most common soft tissue sarcoma of childhood, has an overall cure rate of only 50%. The identification of biologic subgroups of patients with a high risk of failing conventional therapy is critical to the improvement of survival rates for this disease. Morphologic evaluation of these tumors has defined two subgroups, embryonal and alveolar, which correlate with favorable and unfavorable outcome, respectively. However, the morphologic subclassification of these tumors remains difficult. Cytogenetic analysis of alveolar rhabdomyosarcomas has demonstrated the presence of a reciprocal translocation between band q35 on chromosome 2, and q14 on chromosome 13. At a molecular level this translocation involves the disruption of the *PAX3* gene on chromosome 2 and its fusion to a novel member of the forkhead family of transcriptional factors, (*FKH*), on chromosome 13. Utilizing oligonucleotide primers from sequences of *PAX3* and *FKH* bracketing this breakpoint, we have established a reverse transcription polymerase chain reaction (RT-PCR) assay for the detection of the der(13) encoded *PAX3/FKH* fusion transcript. Application of this assay to 34 cases of rhabdomyosarcoma resulted in the amplification of a PCR products of the identical size and sequence in 20/20 cases with an alveolar histology and in only 1/14 cases with a diagnosis of embryonal rhabdomyosarcoma. No evidence of a fusion product was detected in tumors having a diagnosis other than rhabdomyosarcoma. The combination of this RT-PCR assay with oligonucleotide primers for the detection of the Ewing's sarcoma t(11;22) encoded *EWS/FLI-1* chimeric transcript, results in a multiplex RT-PCR reaction which can be routinely used for the accurate diagnosis, staging, and monitoring of these tumors.

D 603 REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) FOR DETECTION OF t(4;11)(q21;q23) IN ACUTE LYMPHOBLASTIC LEUKEMIA (ALL). David Head, Mary G. Hulshof, Anita M. Curcio-Brint, Tina A. Motroni, Peter Domer, James R. Downing, Departments of Pathology and Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN, and Department of Pathology, Northwestern University Medical School, Chicago, IL.

The genes involved in the t(4:11) translocation have recently been identified. Using oligonucleotide primers derived from the *MLL* gene on chromosome 11 and the *AF-4* gene on chromosome 4 we established an RT-PCR reactions for detection of the fusion transcripts from both the der(11) and der(4) chromosomes. Utilizing this assay we have analyzed 23 cases of pediatric t(4;11) containing ALL. Fusion transcripts derived from the der(11) chromosome (*MLL/AF-4*) were detected in each case, with the assay demonstrating a sensitivity of one leukemic cell in 1 x 10^5 non-malignant cells. Sequence analysis of representative products revealed varying fusion mRNAs resulting from breaks in *MLL* introns 6, 7, or 8, with alternative splicing to one of three exons in *AF-4*. In contrast, fusion transcripts derived from the der(4) chromosome were detected in only 60% of the cases analyzed, suggesting that the critical derivative for transformation is chromosome 11. We also evaluated multiplex RT-PCR for t(4;11) (*MLL/AF-4*), t(1;19) *(E2A/PBX*), and t(9;22) (*BCR/ABL*), using *c-ABL* as a control for RNA integrity. RNA extracted from leukemic cell lines containing appropriate translocations was amplified in multiplex RT-PCR reactions, slot blotted, and hybridized to oligonucleotide probes for *PBX*, *ABL*, or *AF-4*. Compared to individual RT-PCR reactions, multiplex RT-PCR resulted in excellent and specific amplification of appropriate products with all 4 primer sets. These data demonstrate the utility of RT-PCR assay for the primary diagnosis of t(4;11) ALL, and the usefulness of a multiplex RT-PCR assay for the primary diagnosis of the major ALL associated translocations.

D 604 Use of Comparative Genomic Hybridization to Study Genomic Instability in Neoplastic Cells, Helene Roelofs, Steve Lockett, Brian Herman, Joe W.Gray and Thea D.Tlsty, Lineberger Comprehensive Cancer Center, UNC, Chapel Hill and Department of Laboratory Medicine, Div. of Molecular Cytometry, UCSF, San Francisco

Comparative genomic hybridization (CGH) is a powerful new technique that combines the widely used fluorescence in situ hybridization technique and differential DNA screening. In addition, this technique requires only a very small number of cells. It can compare the genetic contents of a specific cell population with that of a normal diploid cell population and reveal *whether* this cell population has a deviating DNA dosage (i.e. contains deletions, chromosome losses, amplifications or chromosome gains) and if it does, the technique also shows *which* chromosome or genomic locus is deleted or amplified.

Tumor cells usually show aneuploidy (gains and losses) for multiple chromosomes and also show deletions and amplifications for multiple genomic loci. Most studies performed thus far have documented these kinds of arrangements *in vitro* (in tumor cell lines or cultured tumor cell populations). However, to determine the biological importance of these rearrangements, it is necessary to investigate *in vivo* specimens. Studies that looked at *in vivo* specimens have basically been focussed on specific loci that were already known or suspected to contain oncogenes or tumor suppressor genes. To detect *new* biologically important rearrangements it would be preferable to look at the whole genome (i.e. pangenomically) at once.

With the new CGH technique it has become possible to look pangenomically *in vivo* . We apply this technique not only to look at *in vivo* tumors samples but also at (pre)neoplastic lesions, providing us with information about rearrangements that accompany different tumorigenetic stages.

D 605 FUNCTIONAL ROLES OF AND LOCALIZATION OF CHROMOSOME 10 DELETIONS IN HUMAN GLIOBLASTOMAS Peter A. Steck, Azra Hadi, Mark A. Pershouse, W. K. Alfred Yung, and Paul Cheong, Department of Neuro-Oncology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

The loss of alleles on chromosome 10 is the most common genetic alteration identified in the development of glioblastomas. Utilizing microcell-mediated chromosomal transfer, we have inserted fragments or an entire copy of chromosome 10 into two independent glioblastoma cell lines. The presence of an entire copy of chromosome 10 suppressed dramatically the transformed and tumorigenic phenotype of the glioma cells as assessed by growth in soft agar and tumor formation in nude mice. The hybrid cells also exhibited an 'astrocytic-like' morphology under certain culture conditions, although their exponential growth rate was not significantly altered. To further examine the effects of reinsertion of chromosome 10, subtractive hybridization was performed between cDNA from a hybrid cell with an intact chromosome 10 and mRNA from the parental glioma cells. Presently, several differentially expressed gene products have been isolated, representing both known and novel sequences, and these gene products are being further characterized. To determine the suppressive region of the chromosome, several subclones were selected for their ability to grow under anchorage-independent conditions. These subclones were observed to contain a fragmented chromosome 10 with deletions on the long arm of the chromosome. However, the hybrid cells failed to form tumors. These results combined with previous molecular results suggest the possible presence of two phenotypically suppressive regions on chromosome 10.

D 606 STUDY THE OVERPRODUCTION OF ARGININO-SUCCINATE SYNTHETASE IN CANAVANINE-RESISTANT CELLS, Ting-Fen Tsai[1] and Tsung-Sheng Su[1,2], [1]Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical College and [2]Department of Medical Research, Veterans General Hospital-Taipei, Taiwan, Republic of China.

Argininosuccinate synthetase catalyzes the conversion of citrulline and aspartate to argininosuccinate. The enzyme is present in all tissues and cultured cells studied, but the highest enzyme activity is in liver where the enzyme functions in the urea cycle to eliminate ammonia. A canavanine-resistant variant of human epithelial cell line RPMI 2650 has shown to have elevated level of argininosuccinate synthetase. In one of the variant, Can^{r}1, its enzyme level is abount 200-fold higher than parental cells and reaches the levels in liver. The overproduction does not involve gene amplification and this phenotype is stable when cells are grown in nonselective tissue culture medium. To elucidate the molecular mechanism involved, run-on and run-off transcriptional assays were performed. Using run-on assay, the overproduction in Can^{r}1 cells is shown to be a post-transcriptional event. Furthermore, by run-off transcriptional assay, the nascent transcripts of argininosuccinate synthetase in parental RPMI 2650 cells were shown to be very unstable. Moreover, to understand whether this post-transcriptional regulation involves a *cis*- or a *trans*-acting mechanism, the mRNAs from allelic argininosuccinate synthetase genes in Can^{r}1 cells were distinguished by an exonic microsatellite dinucleotide polymorphism and the levels of mRNA from each allele of the genes were estimated. The data shows that in Can^{r}1 cells both alleles of the genes are overly expressed. Results from this study suggest that a post-transcriptional *trans*-acting mechanism may involve in overproduction regulation in Can^{r}1 cells.

D 607 REPRESSOR FUNCTION OF CCAAT DISPLACEMENT PROTEIN (CDP) IN MYELOID DIFFERENTIATION IN HL-60 STABLE TRANSFORMANTS. Cristina Tufarelli, Patricia M.-J. Lievens, Janae J. Donady, Stuart H. Orkin and Ellis J. Neufeld. Division of Hematology/Oncology, Children's Hospital, Boston MA. 02115

Determinants of tissue-specific gene expression in hematopoietic cells of myeloid lineage remain to be defined. In studies aimed at understanding control of myeloid gene expression, we have previously isolated and cloned CDP as a possible transcriptional repressor involved in terminal myeloid differentiation. CDP is a ubiquitous 180-190 kD homeodomain protein with homology to the *Drosophila* protein, cut, implicated as a repressor protein in several developmental systems. CDP levels vary inversely with expression of the myeloid cytochrome gene, *gp91-phox*, which is induced with terminal differentiation (Skalnik et al, *J. Biol Chem* **266,** 16736, 1991). To test directly the ability of CDP to function as a transcriptional repressor of *gp91-phox* in myeloid differentiation, we have isolated HL-60 promyelocytic leukemia cell lines stably expressing exogenous CDP cDNA in the expression vectors RC-CMV (neomycin^R) and pEF-hygro (hygromycin^R). Stable transformants carrying vector alone (controls) or expressing CDP cDNA were induced to differentiate toward monocyte-macrophage lineage with phorbol ester (PMA), or toward neutrophil lineage with DMSO or retinoic acid/dimethylformamide (RA/DMF). Northern blot analysis was used to assess induction of *gp91-phox*, as well as the cytoplasmic oxidase components, p47 and p67 (which are not thought to be controlled by CDP). In the CDP+ HL-60 stable transformant, but not vector-only controls, *gp91-phox* induction with PMA or DMSO was markedly diminished. Morphologic differentiation, and induction of p47 and p67 mRNA expression were normal in the stable transformants. *gp91-phox* induction by RA/DMF in CDP+ transformants was also diminished, but to a lesser degree than with PMA or DMSO. This observation provides the first direct evidence that constitutive expression of CDP acts to repress the gp91-phox gene, but is not sufficient to prevent other aspects of terminal myeloid differentiation.

D 608 QUANTIFICATION OF *XRCC1* DNA REPAIR GENE EXPRESSION, Christi A. Walter[1], Jianwei Lu[1], Mukesh Bhakta[1], Zi-Qiang Zhou[1], Allegra Broft1[1] and Larry H. Thompson[2], [1]Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284, [2]Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550

Failure to repair DNA damage results in genetic aberrations that are important events in the process of carcinogenesis. DNA repair is also likely to play a role in heritable genetic diseases as evidenced by the high new mutation rate for some syndromes such as neurofibromatosis and Duchenne muscular dystrophy. The human X-ray repair cross complementing 1 (*XRCC1*) gene is implicated in DNA repair based on its ability to restore the parental phenotype when transfected into the DNA repair deficient Chinese hamster ovary cell line EM9 (Thompson et al., 1990, *Molec. Cell. Biol.* 10:6160-6171).

We have developed and utilized a quantitative RNase protection assay to quantify *XRCC1* transcripts in select mouse and baboon tissues. A ^{3}H-labeled and truncated *XRCC1* sense RNA is synthesized *in vitro* and added in known amounts to tissues when they are homogenized for RNA isolation. Immediately after homogenization, an aliquot of each sample is removed for DNA quantification. The samples are then processed for poly (A^+) RNA and subjected to RNase protection assay procedures. A ^{32}P-labeled antisense probe is utilized that hybridizes to the sample *XRCC1* RNA and to the ^{3}H-labeled *XRCC1* truncated RNA. Both hybridizing bands are counted with a Betagen betascope. The ^{3}H-labeled RNA is used as an internal standard to calculate the number of *XRCC1* transcripts per microgram of DNA or per cell. Expression of *XRCC1* is low but present in all tissues tested including brain, heart, liver, lung, skin, spleen, stomach, testis, thymus and embryonic brain and liver. Testes *XRCC1* expression is approximately five times higher than any other tissue tested in mouse and baboon. *XRCC1* expression in testes from mice deficient in germ cells is lower than expression in normal testes thereby indicating that germ cells and somatic cells are synthesizing *XRCC1* RNA in the testis.

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Late Abstract

SECOND GENERATION SICKLE TRANSGENIC MOUSE: THE EXPRESSION OF BOTH β^{S} AND $\beta^{S\text{-Antilles}}$ IS MORE SEVERE THAN EITHER ALONE. R.L. Nagel, F. Costantini, E.M. Rubin, and M.E. Fabry. Division of Hematology, Albert Einstein College of Medicine, Bronx, NY, Department of Genetics and Development, Columbia U, NY, NY and Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, Berkeley, CA

We have crossed a previously characterized transgenic mouse line which expresses high levels of human α- and β^{S}-chains (homozygous β^{Major} deletional background with 72.7±2.4 % β^{S} of all β, $\alpha^{H}\beta^{S}[\beta^{MDD}]$) with a transgenic line expressing human α and $\beta^{S\text{-Antilles}}$. We hypothesized that the resulting mice expressing both hemoglobins would have a more severe phenotype because the reduced oxygen affinity and solubility of the $\beta^{S\text{-Antilles}}$ might increase the chances of polymerization. We have obtained mice which express both transgenes and are either hetero (β^{MD}) or homozygous (β^{MDD}) for deletion of mouse β^{Major}. The β^{MDD} mice expressing both transgenes were under-represented at birth suggesting that they might be partial lethals. Hematocrits, Hb, MCH were normal for all mice, but reticulocytes were elevated for the doubly transgenic mice vs $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice (7.9±2.0% vs 4.2±0.9, $p<0.01$) and control mice (2.7±0.4%). Compensated hemolysis was more severe in newborn mice less than 30 days old [retics 22% (β^{MDD}) and 10% (β^{MD})]. The MCHC was comparable to that of $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice but higher than that of C57BL/6J control mice. Reduced urine concentrating ability is a characteristic of sickle cell disease (SCA) and urine osmolarity was reduced 30% under ambient conditions in all mice expressing both transgenes. The $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice do not have a urine concentration defect at room air. Rate of sickling (pH7.4, 37°) at 10 min was 45% in the $\alpha^{H}\beta^{S}/\beta^{S\text{-Antilles}}$ mice and 18% in the $\alpha^{H}\beta^{S}[\beta^{MDD}]$ mice, and at 1 minute, 15.1% vs 1%, respectively). Individual cell sickling also demonstrated a decrease in delay times in the second generation transgenics. We conclude that $\alpha^{H}\beta^{S}/\beta^{S\text{-Antilles}}$ mice have a higher tendency to sickle and a more severe phenotype than those expressing a high level of either $\alpha^{H}\beta^{S}$ or $\alpha^{H}\beta^{S\text{-Antilles}}$. These mice are a suitable model for studying urine concentration defect, testing protocols for preserving renal function, and the study of organ damage characteristic of sickle cell anemia patients.