Models of Human Disease

Oral Presentation **Sunday November 6** **9.00am – 9.15am**

O-11

GERMLINE TRANSMISSION OF HUMAN CHROMOSOME 21 IN AN ANEUPLOID MOUSE STRAIN WITH DOWN SYNDROME PHENOTYPES

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At least 5% of all human pregnancies are aneuploid, and ~1 in 700 children are born with Down syndrome (DS) which results from having three copies of human chromosome 21 (Hsa21). DS is the most common known genetic cause of mental retardation and also results in increased susceptibility for other disorders, including developmental deficits. It is a complex disorder that involves multiple Hsa21 genes in interaction with the rest of the genome. To gain insight into the biology of DS, we have generated a new type of mouse model in which an almost complete human chromosome, Hsa21, segregates through the germline. We present evidence that this trans-species aneuploid mouse strain, 'Tc1', displays phenotypic alterations in behaviour, synaptic plasticity, cerebellar neuronal number and heart development that relate directly to human DS and to other partial trisomy models of DS. Transchromosomic mouse lines such as Tc1 may represent useful genetic tools to dissect other aneuploidies and complex human genetic conditions.

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Oral Presentation **Sunday November 6** **9.15am – 9.30am**

O-12
CELL-AUTONOMOUS MITOGENESIS RESPONSE DEFECT TO HEDGEHOG SIGNALING IN DOWN SYNDROME MICE

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Ts65Dn mice are trisomic for orthologs of about half of the genes on human chromosome 21 and display a number of developmental anomalies analogous to those in Down syndrome. In particular, we showed that alterations in the cerebellum of trisomic mice mimic the pathology of Down syndrome, including a reduced number and density of granule cell neurons in Ts65Dn mice which correctly predicted a corresponding phenotype in humans with Down syndrome. We have traced the granule cell deficit to the earliest point at which trisomic and euploid cerebella diverge, identified granule cell precursors (gcp) as the affected cells and shown that the response of gcp to the effects of sonic hedgehog (Shh) growth factor-induced mitogenic pathway underlies inadequate generation of granule cells. Trisomic gcp have an intrinsic deficit in response to Shh, showing a reduced but dose-dependent response to Shh protein in vitro. These results suggested that increasing local Shh concentrations might overcome all or part of this deficit in vivo. Systemic treatment of newborn trisomic mice with a small molecule agonist of Hedgehog pathway activity increased mitosis and restored granule cell precursor populations in the critical developmental period immediately after birth. This is the first report of amelioration of a neuronal deficit in Down syndrome, and identifies a target for possible clinical interventions in a central component of cognitive disability in trisomy 21.

Models of Human Disease

Oral Presentation **Sunday November 6** **9.30am – 9.45am**

O-13

ENU MUTAGENESIS AND THE PHENOTYPE-DRIVEN APPROACH: IS IT WORTH THE EFFORT?

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Several large-scale mouse mutagenesis programs have been established in the last decade in an effort to accelerate our understanding of gene function and ultimately lead us to better management of human disease. These programs are largely based on strategies developed in other model organisms such *C. elegans*, *D. rerio*, and *D. melanogaster*. In translating this approach to the mouse, an enormous commitment of resources is required. We report our progress in the Heart, Lung, Blood and Sleep Mutagenesis Program at The Jackson Laboratory, initiated in late 2000. We developed novel, high-throughput, robust primary screens designed specifically for mice that have been validated by more invasive means and by other laboratories. We have established over 60 heritable mutants and have identified new alleles and functions for known genes. Many other models are currently in heritability testing. We have mapped a subset of our new mutants and will report on the varied success in mapping including resultant QTLs when genetic backgrounds are mixed. Our diverse protocol is easily extended to screening existing knockout, transgenic and inbred strains. We have screened 43 inbred strains using our protocol, providing valuable phenotype information for choosing strains to use for mapping. Whole-animal mutagenesis followed by high throughput phenotyping is clearly an effective, unbiased tool for identifying genes underlying human disease.

Models of Human Disease

Oral Presentation **Sunday November 6** **9.45am – 10.00am**

O-14

REVERSE GENETICS BY ENU-BASED GENE-DRIVEN MUTAGENESIS IN THE MOUSE

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ENU mutagenesis has been widely used to generate batteries of human disease models. It primarily aims to establish mutant strains by phenotype-driven approach, in particular, to identify yet unknown responsible genes for human diseases. One of the major objectives of the RIKEN mouse mutagenesis project is to identify mouse models for human common diseases. To securely retain useful mutant strains, all the G1 sperm were subjected to cryopreservation. Currently, about 10,000 G1 males have been subjected to the sperm cryopreservation. The G1 frozen sperm archive also provides a mutant mouse library for reverse genetics. To make this gene-driven mutagenesis feasible, 1) corresponding G1 genomic DNA archive construction, 2) PCR primer designing for target genes, 3) high throughput point mutation discovery system and 4) live mouse retrieval are necessary. Currently, we set a genomic DNA archive for ~8,000 G1 mice. We have listed 195 target genes and designed their PCR primers. We firstly used the direct sequencing method to detect ENU-induced mutations and then added Temperature Gradient Capillary

Electrophoresis as a quick pre-screening system. We have identified more than 200 ENU-induced mutations. The molecular characterization revealed the nature of ENU mutagenesis in the mouse genome. For instance, we have identified even a minor fraction of the clonal expansion of a common ancestor mutation in the G0 spermatogenesis. Finally, we have retrieved more than 30 live mouse strains out of ~200 identified mutations from the archive. Some G3 offspring have already been born in several strains and subjected to phenotype screening.

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Oral Presentation **Sunday November 6** **2.00pm – 2.15pm**

O-15

A SENSITIZED ENU MUTAGENESIS SCREEN FOR GENETIC MODIFIERS OF RHEUMATOID ARTHRITIS AND INFLAMMATORY BOWEL DISEASE

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Genome-wide, random mutagenesis with the ethylating mutagen N-ethyl-N-nitrosourea (ENU) has become an attractive method to track the role of virtually any gene in a particular phenotype. In particular, ENU mutagenesis of disease sensitized animals offers unique opportunities to discover gene functions directly associated with prevention or therapy of diseases. We have thus initiated a Program of sensitized ENU mutagenesis screen applied on our established $TNF^{\Delta ARE}$ model of arthritis and Crohn's-like inflammatory bowel disease (IBD), to identify modifier gene candidates associated with development of these diseases. By using simple and accurate phenotypic screens, clinical score for arthritis and macroscopic or histological analysis for IBD, we are selecting the individual progeny that display disease attenuation. By screening 4304 G3 offspring we have identified 3 families which show significant delay on the onset and progression of arthritis and 2 families with dramatic attenuation of IBD. In parallel to the sensitized screens we have also identified novel recessive phenotypes ie. a mouse mutant model which shows severe osteopetrosis, defect in tooth eruption and complete lack of osteoclasts.

A genetic mapping approach is currently in progress which involves an F2 intercross mapping scheme and genome-wide microsatellite typing, in order to identify potential susceptibility loci. Initial mapping efforts have already identified candidate chromosomal regions for specific mutants, whereas fine mapping using SNPs analysis is currently underway. Once identified these novel gene functions may constitute validated pharmaceutical targets for the treatment of chronic inflammatory disease.

Models of Human Disease

Oral Presentation **Sunday November 6** **2.15pm – 2.30pm**

O-16

DOMINANT MUTATIONS OF COL4A1 RESULT IN BASEMENT MEMBRANE DEFECTS WHICH LEAD TO ANTERIOR SEGMENT DYSGENESIS AND GLOMERULOPATHY

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Members of the type IV collagen family are essential components of all basement membranes and define structural stability as well as tissue-specific functions. The major isoform, a1.a1.a2(IV), contributes to the formation of many basement membranes and its deficiency causes embryonic lethality in mouse. We have identified an allelic series of three ENU induced dominant mouse mutants with missense mutations in the gene *Col4a1* encoding the α1(IV) subunit chain. Two severe alleles (*Bru* and *Svc*) have mutations affecting the conserved glycine residues in the Gly-Xaa-Yaa collagen repeat. *Bru* heterozygous mice display defects similar to Axenfeld-Rieger anomaly including iris defects, corneal opacity, vacuolar cataracts, significant iris/corneal adhesions, buphthalmos and optic nerve cupping, a sign indicative of glaucoma. Kidneys of *Bru* mice have peripheral glomerulopathy characterised by hypertrophy and hyperplasia of the parietal epithelium of Bowman's capsule. A milder allele (*Raw*) contains a mutation in the Yaa residue of the collagen repeat and was identified by a silvery appearance of the retinal arterioles. All phenotypes are associated with basement membrane defects that affect the eye, kidney and other tissues. This allelic series shows that mutations affecting the collagen domain cause dominant negative effects on the expression and function of the major collagen IV isoforms α1(IV) and pathological effects vary with the individual mutations.

Models of Human Disease

Oral Presentation **Sunday November 6** **2.30pm – 2.45pm**

O-17

ALLELIC SERIES IN THE MC4R GENE DEMONSTRATES THAT VARYING LEVELS OF OBESITY CORRELATES WITH RECEPTOR FUNCTION

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Defects in the melanocortin 4 receptor (*Mc4R*) have a well-established role in obesity in both humans and mice. Herein, we report the isolation of an allelic series through ENU mutagenesis in the mouse *Mc4R* gene that mimics the obesity found in human patients. As in humans, the severity of the obesity phenotype is directly related to the amount of function remaining for each receptor. One missense mutation, South Beach, fails to translocate to the surface of the cell in *in vitro* assays and, therefore, has no receptor activity. A second missense receptor mutation, Fat Boy, has signaling properties similar to the wild type Mc4R even though it has only 14% of the surface expression levels as measured by specific binding. Both mutant mice display obesity although the South Beach mice are significantly heavier. Similar to the *Mc4R* knockout mice and humans carrying *Mc4R* mutations, mice heterozygous for the mutations described here display an intermediate level of obesity as compared to control littermates. This effect demonstrates the sensitivity of this receptor such that partial haploinsufficiency still yields a discernable phenotype. These mutant mice will serve as good models for the variation in obesity found in humans with mutations in Mc4R.

Models of Human Disease

Oral Presentation **Sunday November 6** **2.45pm – 3.00pm**

O-18

MUTANT MOUSE MODELS OF NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE SHOW DEFECTS IN INSULIN SECRETION AND GLUCOSE TOLERANCE

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Insulin release from pancreatic beta-cells is regulated by glucose metabolism. When plasma glucose levels rise, intracellular ATP levels increase, and close ATP-sensitive potassium (K_{ATP}) channels. This results in membrane depolarisation, activation of voltage-gated Ca^{2+} channels, influx of Ca^{2+} and exocytosis of insulin-containing vesicles.
The C57BL/6J mouse displays glucose intolerance and reduced insulin secretion. QTL mapping identified Nicotinamide Nucleotide Transhydrogenase (*Nnt)* as a strong candidate gene. *Nnt* is a nuclear-encoded mitochondrial protein thought to be involved in free radical detoxification.

We identified two ENU-induced point mutations in *Nnt* (N68K, G745D). *Nnt* mutant mice were glucose intolerant and secreted less insulin during a glucose tolerance test. Isolated islets also showed impaired insulin secretion in response to glucose, but not to tolbutamide. This was a consequence of reduced ATP generation at elevated glucose in *Nnt* mutant islets. We also used siRNA to knock down *Nnt* in the insulin-secreting cell line MIN6. This resulted in a dramatic reduction in insulin secretion and in the rise in $[Ca^{2+}]_i$ evoked by glucose, but not elicited by the sulphonylurea tolbutamide. Both applications therefore confirmed the functional role of *Nnt* in insulin release.

We hypothesise that *Nnt* mutations impair beta-cell mitochondrial metabolism which thereby accounts for the lower ATP production, and enhances the activity of K_{ATP} channels. Consequently, glucose-dependent beta-cell electrical activity and insulin secretion are impaired. This in turn leads to impaired glucose tolerance in the animal.

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Oral Presentation **Sunday November 6** **3.00pm – 3.15pm**

O-19

FILAMIN B MUTATIONS CAUSE CHONDROCYTE HYPERTROPHY DURING SKELETAL DEVELOPMENT

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Mutations in *Filamin B*, a gene encoding a cytoplasmic actin binding protein, have been found in five human skeletal disorders: boomerang dysplasia, spondylocarpotarsal syndrome, atelosteogenesis I, atelosteogenesis III and Larsen syndrome. To study the role of Filamin B in mammalian skeletal development, we generated mice using an ES cell line trapped with a β-galactosidase-neomycin resistance fusion gene. The mice lacking the full-length protein display ectopic ossification of various bone elements, most prominently in the cervical and thoracic vertebrae, sterna, chondracostal cartilage and carpal bones. The phenotypic abnormalities mimic those of the human skeletal disorders with nonsense mutations in *Filamin B* gene. The aberrant bone formation is due to ectopic chondocyte hypertrophy, which can also be caused by loss of histone deacetylase 4 or constitutive expression of Runx2 in chondrocytes. Together with our previous finding that filamin B may interact with *Odz4*, a transmembrane receptor implicated in skeletogenesis, these results suggest that Filamin B mediates a signaling cascade controlling chondrocyte hypertrophy during bone development.

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Oral Presentation **Sunday November 6** **3.15pm – 3.30pm**

O-20

GENETIC INTERACTION BETWEEN *ITCH* AND *NOTCH1* IN A MOUSE AUTOIMMUNE DISEASE MODEL SUGGESTS A ROLE FOR *NOTCH1* SIGNALLING IN NEGATIVE SELECTION AND CELL SURVIVAL

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Itch represents one of the few single gene mouse autoimmune disease models. Homozygous *itchy* (*itch*) mice develop a progressive disease characterized by systemic inflammation that proves fatal at 6-8 months of age due to congestive pneumonia. This autoimmune-like disease results from a loss of function mutation in a HECT E3 ubiquitin protein ligase. Phylogentic and *in vitro* analyses suggest that Itch is a negative regulator of Notch signalling. To assess the biological role of Itch in Notch signalling, we bred *itch* mice to mice carrying an activated *Notch1* transgene under the control of the *lck* proximal promoter. Interestingly, *itch* mice that carry the *Notch1* transgene are significantly smaller than their littermates and die by 12 weeks of age. They also have the same autoimmune disease seen in *itch* animals; however, the disease is much more severe and develops much sooner. T cell development is also perturbed in these mice, with a reduction in the number of CD4, CD8 double positive cells and an increase in the number of double negative and single positive cells. TUNEL staining shows reduced apoptosis in the thymus of *itch* + *Notch1* transgenic animals and antibody staining for Notch1 displayed increased levels of full length (FL) Notch1 in the thymus but not in the spleen. Collectively, these results demonstrate that *Itch* and *Notch1* synergize in this autoimmune-like disease, and suggest that increased Notch1 signalling through increased FL Notch1 in thymocytes provides a survival signal to cells that were otherwise fated to die after negative selection.

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Oral Presentation **Sunday November 6** **3.30pm – 3.45pm**

O-21

DISCOVERY OF GENES AND PATHWAYS ASSOCIATED WITH DISEASE BY THE MOUSE ENU-MUTAGENESIS APPROACH

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We, as a team of physicians (cardiologists, neurologists, metabolic specialists), biomedical scientists, and human and molecular geneticists, established a comprehensive mouse clinic in which highly sophisticated and specialized screening tests for disease phenotypes can be performed. The overall goals of our programs are to unravel genes and pathways associated with human diseases and to generate mouse models for them.

Using a genome-wide mutagenesis approach with *N*-ethyl-*N*-nitrosourea (ENU) to produce mutant mice, followed by phenotype screening and high-throughput genotyping to localize the mutations, we have identified mouse models of neurological diseases (brain atrophy, necrotizing encephalopathy, hydrocephalus, pain hyper-sensitivity, pentobarbital resistance), cardiovascular diseases (aortic stenosis, cardiac arrhythmia), renal disease (hydronephrosis), hematology (lymphopenia) and metabolic diseases (human maple syrup urine-like disease and fatty acid oxidation defects). These mouse models are unique, as most of them have not been reported from ENU programs elsewhere. We have confirmed the heritability and performed whole genome homozygosity mapping (MassARRAY SNP genotyping platform) using a panel of 299 SNPs on most of these models.

In the cases of the mouse models resembling human maple syrup urine disease, fatty acid oxidation defects, and lymphopenia, the responsible genes causing the diseases have been identified. We will continue to identify more mouse models with distinct phenotypes. Insights obtained from characterizing novel genes and pathways of human diseases and the mouse models generated will not only enhance our understanding of the patho-physiology of the diseases but also improve the diagnosis and facilitate the development of more effective therapies.