

The background of the entire page is a photograph of a city skyline at sunset. The sky is a deep orange and red, with the sun low on the horizon. The city buildings are silhouetted against the sky. In the foreground, there is a body of water that reflects the colors of the sky and the buildings. The overall mood is serene and scenic.

Monday November 7

3.00pm – 5.30pm

Poster Session 2

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Models of Human Disease

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¹ The Jackson Laboratory, Bar Harbor, ME, United States, ² University of Alberta, Edmonton, Alberta, Canada
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M Szatanik¹, J Jaubert¹, N Martin¹, C De Waele², I Vassias², J L Guenet¹
¹ Institut Pasteur, Paris, France, ² CNRS Université Paris 5, Paris, France
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 GSF - National Research Center for Environment and Health, Neuherberg, Germany
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A Acevedo Arozena¹, B Ravikumar², D C Rubinsztein², S D M Brown¹
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F Achilli¹, Z Lalanne², D Brooker², A Hardy², K Strand¹, T Revesz¹, J Holton¹, P Nolan², E Fisher¹
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 R Rosenkranz, B Greber, A Ludewig, H Tandara, H Lehrach, H Himmelbauer
 Max-Planck-Institute for molecular Genetics, Berlin, Germany
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A Schrewe¹, B Ivandic¹, P Kirchhof², J Stypmann², V Gailus-Durner³, M Hrabe-de Angelis³, H Katus¹
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- P-48 GENETIC MODIFIERS OF NEURODEGENERATION IN MICE LACKING THE NIEMANN-PICK TYPE C (*NPC2*) GENE**
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- P-51 GEMIN2 PLAYS AN IMPORTANT ROLE FOR STABILIZATION OF THE SMN COMPLEX**
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M Ahmed, A Loualich, M Al-Buhairi, J Yang, R Strome, D Westaway, G Alsbeigh, M A Chishti
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Z Hossain, S M Ali, X Jianliang, G Ke, W Hunziker
 Institute of Molecular and Cell Biology, Singapore 138673, Singapore
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 Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan
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R Kominami, Y Mishima, k Kamimura, M Obata
 Medicine and Dentistry, Niigata, Japan
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M Inoue¹, H Motegi¹, Y Sakuraba², H Toki¹, J Matsui¹, T Hirayama¹, H Kaneda¹, H Masuya¹, S Wakana¹, Y Gondo², O Minowa¹, T Shiroishi¹, T Noda¹
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V Gailus-Durner
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 S Brown, H Gates
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The Jackson Laboratory, Bar Harbor, Me, United States
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Nokad, Evry, France
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M I Lomax, T W L Gong, G Dootz, L Fullarton, K Hunker, C A Lomax, D F Dolan, R A Altschuler, D C Kohnman
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K Fujiwara, J Igarashi, H Nagase
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B Yang, P Shi, E Sweezer, S Guo, T Kinney, X Cao
University of Iowa, Iowa City, Iowa, United States
- P-69 THREE MOUSE MODELS FOR HUMAN HYPOPHOSPHATEMIC RICKETS GENERATED BY A LARGE SCALE N-ETHYL-N-NITROSOUREA MUTAGENESIS PROJECT**
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- P-70 A NEW MUTANT MOUSE (SLW) WITH ACHONDROPLASIA CAUSED BY A MUTATION IN CNP RECEPTOR GENE**
C Sogawa, Y Shinkai, T Tsuji, T Kunieda
Okayama University, Okayama, Japan
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J Demassue¹, J Demassue³, A Hanauer², A Bloch-Zupan¹, A Bloch-Zupan³
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T Yasuda, H Yoshida
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S H Cross¹, J E Morgan¹, A W Hart¹, K West¹, L McKie¹, J E Schneider², S Bhattacharya², I J Jackson¹
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C Rudolph¹, M Braig², C Bittner³, CA Schmitt², H Merz³, B Schlegelberger¹
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M Hagn, G Tocchini-Valentini, Y Herault, S Brown, L Aehrlund Richter, M Mallo, J Thornton, M Hrabec de Angelis
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Y Kikkawa¹, H Shitara¹, H Yonekawa¹, A Mita², T Shiroishi², Y Morita³, M Nemoto³, R Kominami¹
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S S M Malhotra, E L T Travis
 University of Texas MD Anderson Cancer Center, Houston, TX, United States
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 T Epp¹, T S Tanaka¹, C Y J Li¹, T Reid¹, Q Lan¹, M Yu¹, Q Morris¹, C To¹, A Nagy², TR Hughes¹, J Rossant³, WL Stanford¹
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R J Westrick¹, S L Manning², S L Dobies¹, M E Winn¹, D R Siemieniak², L M Korepta¹, D Ginsburg¹
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- P-88 MOUSE MODELS OF HUMAN DISEASE: THE JACKSON LABORATORY REPOSITORY**
D B Lane, S F Rockwood, C M Lutz, L R Donahue, M T Davisson
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J S Weber¹, G Schnell², A Kreis¹
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L N Kwong, A Shedlovsky, W F Dove
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C Yildiz, R Ayearst, N Law, L Liu, P Ottaviani, C McKerlie
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- P-92 A NEW MOUSE MUTANT FOR THE LDL RECEPTOR**
K L Svenson¹, N Ahituv², B Paigen¹, L L Peters¹
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M A Bailey, C J Kenyon, S Thakrar, J J Mullins, L J Mullins
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B Rathkolb¹, J Calzada-Wack², M Klasten³, S Kalaydjiev⁴, M Mohr¹, T Franz⁴, S Wagner³, D Busch⁴, L Quintanilla-Martinez², B Aigner¹, M Hrabé de Angelis³, E Wolf¹
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- P-96 ROLE OF AP-2g IN ERBB-2-INDUCED TUMORIGENESIS**
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P-97 *STUDENT ORAL ABSTRACT S-3***INTRONIC INSERTION OF A SHORT ENDOGENOUS RETROVIRAL LTR FRAGMENT IN MGLUR1 GENE DISRUPTS MRNA SPLICING AND CAUSES ATAXIA IN CRV4 MOUSE MODEL**

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P-98 *STUDENT ORAL ABSTRACT S-4***DEVELOPMENT AND IMPLEMENTATION OF A VIABLE, PHARMACOLOGICALLY SENSITIZED ENU SCREEN TO IDENTIFY HEMATOPOIETIC MUTANTS IN THE MOUSE**

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P-99 *STUDENT ORAL ABSTRACT S-5***REGION SPECIFIC ENU MUTAGENESIS ON MOUSE CHROMOSOME 5**

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P-100 *STUDENT ORAL ABSTRACT S-6***A GERMLINE TRUNCATING MUTATION IN THE RAT ADENOMATOUS POLYPOSIS COLI GENE WITHIN THE HUMAN MUTATION HOTSPOT REGION**

L N Kwong, J M Amos-Landgraf, K Chen, JD Haag, JL Waller, J L Remfert, Y Zan, M N Gould, W F Dove
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P-101 *STUDENT ORAL ABSTRACT S-7***PHENOTYPIC AND MOLECULAR CHARACTERISATION OF THE DEPILATED (DEP) HAIRLOSS MUTATION**

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P-102 *STUDENT ORAL ABSTRACT S-8***CHARACTERISATION OF SHORTY, AN ENU DERIVED MUTANT MOUSE WITH DEFECTS IN RIB FORMATION**

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A ROSTROCAUDAL MUSCULAR DYSTROPHY CAUSED BY A DEFECT IN MEMBRANE PHOSPHOLIPID BIOSYNTHESIS

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In August 2001, mice in a colony at The Jackson Laboratory developed a muscular dystrophy (MD) resulting from a spontaneous recessive mutation on Chromosome 15. The disease is observable around birth with a disordered forelimb bone development, and mice rapidly develop a progressive rostral to caudal muscular dystrophy. The causative gene is Choline Kinase beta (Chkb), which catalyzes the first step of the production of phosphatidylcholine. Of the two previously described forms of MD membrane defects, the first results in inherent instability of the membrane through faulty structural proteins in the dystrophin-glycoprotein complex. The second is due to compromised membrane repair due to defects in the dysferlin protein. Our MD model involves a gene involved in the production of one of the major constituents of muscle cell membranes, and may therefore represent a novel pathway for muscle degeneration. Potential therapies for muscular dystrophies depend upon detailed knowledge of both the genetic and functional causes of the diseases. New animal models such as this may assist in the discovery of novel mechanisms of disease progression, and therefore may aid in the development of therapeutic treatments. Understanding the biology of this mouse line may provide important insight into the biological pathways leading to muscle development, muscular dystrophy and muscle repair.

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CHARACTERIZATION OF A NOVEL GENE THAT IS A CANDIDATE FOR THE DISEASE LOCUS IN THE CY RAT MODEL OF POLYCYSTIC KIDNEY DISEASE

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Polycystic kidney disease (PKD) is one of the primary causes of end-stage renal disease in both adults and children. A number of rodent models of PKD exist including the Han:SPRD-Cy model which is the only rat model with an autosomal dominant mode of inheritance. This model has long been a focus of study since the slow progression of cystic disease, the autosomal dominant mode of inheritance and the gender bias towards males having more disease severity all closely mimic important aspects of human autosomal dominant (AD) PKD. It is surprising, therefore that while the Cy locus has been localized to rat Chromosome 5 (Bihoreau et al., 1997), the gene has not been cloned. We have begun characterization of a novel gene on rat Chromosome 5 that is tightly linked to disease phenotype as assessed by ultrasound, BUN and histology. Based on comparative sequence analysis, the human orthologue maps to Chromosome 9 while the mouse orthologue maps to Chromosome 4. This gene contains 18 exons and encodes an mRNA of approximately 3 Kb in size. The predicted protein is 928 amino acids in length and contains two types of functional protein motifs: ten ankyrin repeats and a sterile alpha motif (SAM) domain. A single base pair change that has not been detected in 100 unrelated normal Sprague Dawley animals but has 100% correlation with the disease allele falls within the SAM domain. This single base alteration leads to an amino acid change that would be predicted to affect the function of the SAM domain in the Cy protein. Current efforts are underway to provide proof that this novel gene is the disease-causing gene in the Cy rat model.

P-42**A SPONTANEOUS DELETION IN *KCNN2* GENE ENCODING THE SMALL-CONDUCTANCE Ca^{2+} ACTIVATED K^{+} CHANNEL SUBUNIT 2 (SK2) LEADS TO CONSTANT TREMOR IN THE *FRISSONNANT* MOUSE**

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A mouse mutation with locomotor instability and constant tremor was identified in a C3H breeding stock in 1989 at the Institut Pasteur. This phenotype was found to be caused by a recessive mutation, designated *frissonnant* (*fri*), that mapped to the proximal region of Chromosome 18. A detailed analysis of *fri/fri* mice phenotype revealed no impairment of sensitivity or thermoregulation, no anatomopathological lesions of the nigrostriatal systems, motor behavior dysfunctions (abnormal gait, decrease in locomotor activity and decreased performances with rotarod test) and some memory deficits. In most central neurons, action potentials are followed by an after-hyperpolarization (AHP) that controls firing patterns and excitability. Small-conductance Ca^{2+} -activated potassium channels (SK channels) are heteromeric complexes of SK α -subunits and calmodulin, which modulate membrane excitability and are responsible for the AHP. To-date, three SK channel subunits have been molecularly characterized, respectively SK1 to SK3. The *Kcnn2* gene coding the SK2 channel subunit mapped in the vicinity of the *fri* mutation and thus was a candidate gene. A null allele for *Kcnn2* gene was recently reported and mice displayed AHP defects as expected but no gross morphological abnormalities. In the *frissonnant* mouse, we observed a spontaneous deletion in the *Kcnn2* gene resulting in a brain-specific new splice isoform that could explain the motor behavior and constant tremor phenotype.

P-43**THE MUNICH ENU MOUSE MUTAGENESIS PROJECT: A HIGH THROUGHPUT MAPPING STRATEGY IN A LARGE-SCALE MUTAGENESIS SCREEN**

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To date more than 680 recessive and dominant mutant mouse lines have been established in the genome-wide large-scale screen, out of which 75 lines have been mapped and 28 lines have been cloned. Considering this number, a systematic high-throughput mapping of all available mutant mouse lines has become indispensable.

Therefore a genotyping platform including the automated DNA extraction of mouse tail clips and high-throughput SNP analysis based on MALDI-TOF mass spectrometry has been established.

Additionally, DNA extraction of the F1 archive, containing approximately 7000 F1 sperm samples, has been started. The genotyping of up to 384 individuals can be performed within one week with a panel of 150 SNP markers evenly distributed over the genome.

The Advanced Retrieval Tool for SNPs (ARTS) was designed to facilitate the retrieval of SNPs for the setup of mouse genotyping panels (Klawns M and Hrabec de Angelis M, Nucleic Acids Res 2005).

ARTS has several advantages over the existing search engines in terms of flexibility and comprehensibility of the output. Multiple filters can be applied to improve the quality of the search results. This reduces the number of assays that do not pass in vitro validation.

One example of a successful characterization of a skin pigmentation phenotype and the systematic cloning of its underlying mutation will be given (Fitch GR et al., Genes Dev 2003; Van Raamsdonk CD et al., Nat Genet 2004). Novel genes and map locations involved in pigmentation in dark skin mutant mouse lines could be identified.

P-44**DYNEIN MUTATIONS ENHANCE HUNTINGTON'S DISEASE IN THE MOUSE, IDENTIFYING A NEW MECHANISM OF INCLUSION FORMATION IN NEURODEGENERATIVE DISEASES**A Acevedo Arozena¹, B Ravikumar², DC Rubinsztein², S D M Brown¹¹ MRC Mammalian Genetics Unit, Harwell, United Kingdom, ² Department of Medical Genetics, Cambridge Institute for Medical Research, Cambridge, United Kingdom

Mutations in the dynein motor machinery can cause motor neuron disease but it is not known why inclusions appear in affected tissues in mice and in forms of human motor neuron disease. We therefore investigated the role of the dynein machinery in aggregate formation and toxicity in Huntington's disease (HD). As in other proteinopathies (e.g. Parkinson's disease, Amyotrophic Lateral Sclerosis), the presence of neuronal inclusions is one of the disease hallmarks. We studied the consequences of dynein dysfunction in a transgenic mouse model of Huntington's disease (*HD/+*) by crossing it with the Legs at odd angles (*Loa*) mouse. *Loa* carries an ENU-induced missense mutation in the cytoplasmic dynein heavy chain 1 (*Dnchc1*) gene that results in defective fast retrograde transport and late onset neurodegeneration. We followed the onset and progression of disease in both single and double mutant mice with behavioural and immunohistochemical analysis. HD mice carrying the *Loa* mutation (*HD/+;Dnchc1^{Loa/+}*) presented an earlier onset of behavioural symptoms (including tremor onset, rotarod, grip strength deficits) as well as decreased survival when compared to their HD (*HD/+;Dnchc1^{+/+}*) and *Loa* (*+/+;Dnchc1^{Loa/+}*) littermates. Dynein dysfunction in HD mice also resulted in premature aggregate formation and increased levels of the autophagosome marker LC3-II, compatible with impaired autophagosome-lysosome fusion. The results (see *Nature Genetics* 2005 37: 771-776) provide a mechanistic link between dynein mutations and inclusion formation in motor neuron diseases. It appears that dynein mutations impair the clearance of aggregate-prone proteins, possibly due to decreased transport of autophagosomes to lysosomes at perinuclear regions.

P-45**CHARACTERIZATION OF NEW LOCOMOTOR MUTANTS**F Achilli¹, Z Lalanne², D Brooker², A Hardy², K Strand¹, T Revesz¹, J Holton¹, P Nolan², E Fisher¹¹ Department of Neurodegenerative Disease, Institute of Neurology, London, United Kingdom,² UK MRC Mammalian Genetics Unit, Harwell, United Kingdom

The aim of this study was to phenotypically and genotypically characterize three mutant mouse lines (GENA201, 202 and BHV/7) with motor deficits as assessed in the primary screen of the Harwell ENU Mutagenesis Program.

For the purpose of this motor neuron disorder study, we have adopted four parameters to assess the motor performance of the three mouse strains. The parameters measured are (1) grip strength, (2) rotarod performance, (3) wire manoeuvre and (4) weight from ages 1-12 months.

GENA 201 and 202 heterozygous mice showed poor grip strength. The results showed poor correlation between grip strength, rotarod and wire manoeuvre performances. No significant weight loss was observed in the mice.

On the other hand, BHV/7 heterozygous mice were significantly lighter than wild types and showed an ataxic gait with retropulsion. Histopathology data from 6 months old BHV7 mutant mouse brain revealed cerebellar defects characterized by loss of Purkinje cells, gliosis and abnormal dendritic trees.

To identify the mutant genes underlying the GENA201-202 and BHV/7 phenotypes, mutant backcross progeny were genotyped with a panel of markers spanning the genome. Both GENA 201 and 202 mutations have been localised to a 5Mb region on chromosome 6 suggesting being allelic. Genotyping of BHV/7 mutants localised the mutation to a 24Mb interval on chromosome 3. A candidate gene approach will begin once we have narrowed the critical regions to suitably manageable levels.

P-46**MOUSE ES CELL CHEMICAL MUTAGENESIS AS A TOOL FOR FUNCTIONAL GENOMICS**

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Despite the availability of mammalian genome sequences, the functions of many genes remain unknown. The mouse is particularly well suited to address questions concerning gene functions, because the mouse germline is easy to manipulate. Ideally, a mutant resource covering all mouse genes and different mutation types (complete knockout; partial, domain-specific inactivation) would be available. In this respect, our work focusses on the use of ethylnitrosourea (ENU) as a potent mutagen for introducing mutations into the embryonic stem (ES) cell genome. At the molecular level, ENU-induced point mutations cause amino acid replacement, confer premature stop of translation, or disrupt mRNA splicing. Our mutant ES cell clone archive currently consists of 40,000 clones distributed into 96 times 96 samples in a microtiter plate format. The generation of additional archives with ES cell lines with improved properties is in progress. In parallel, we have evaluated mutation detection technologies both for their robustness and applicability for the analysis of pooled samples. The implementation of novel high-throughput technologies for mutation scans within many genes of interest is presently in progress. We have also shown germline competence of the ENU-treated cells, in a pilot project using an ES cell clone isolated by us that constitutively skips exon 18 of the Kit gene, due to the ENU-induced lesion. Taken together, we have assembled valuable tools for the further molecular dissection of mammalian gene function. Concepts and current state of the work will be presented.

P-47**THE GERMAN MOUSE CLINIC – ESTABLISHMENT OF A NEW CARDIOVASCULAR PRIMARY SCREEN**

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The German Mouse Clinic (www.mouseclinic.de) is a national core facility for the comprehensive phenotyping of mice. It is funded by the German National Genome Research Network (www.ngfn.de). Recently, non-invasive cardiovascular phenotyping methods (primary screen) have been established newly to complement the already extensive phenotyping program. Various collaborators contribute genetically manipulated mice (knock-out, transgenic, ENU, gene trap, recombinant inbred) to identify and phenotype novel mouse models for human diseases.

For the primary cardiovascular screen we have established echocardiography (Vevo660, Visualsonics Inc.) and digitized ECG (EMKA Inc.) under isoflurane sedation as well as blood pressure measurements (tail cuff). Interesting phenotypes, identified as a results of the primary screen, are further characterized in secondary and tertiary screens offered by the Cardiovascular Disease Network (www.herz-kreislauf-netz.de) and the Muenster Mouse Clinic. The methods of the primary screen will be adapted to standards used in the EUMORPHIA and NIH NHLBI programs to allow comparability.

P-48**GENETIC MODIFIERS OF NEURODEGENERATION IN MICE LACKING THE NIEMANN-PICK TYPE C (NPC2) GENE**

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Niemann-Pick Disease, Type C (NPC) is a progressive neurodegenerative disorder with onset in early childhood that is rapidly fatal, often before the second or third decade. Symptoms include motor-coordination difficulties, abnormal gait, and cognitive decline. Amazingly, great clinical variability is seen, even between siblings affected with this disease, suggesting that multiple genetic and/or environmental factors affect the onset and progression of neurodegeneration. Two genes (NPC1 and NPC2) have been shown to cause NPC in humans. Mutation in either gene is associated with accumulation of cholesterol in the lysosome and abnormalities in sphingolipid metabolism. Progression of NPC is associated with loss of purkinje cells in the cerebellum, leading to progression of neurologic symptoms. Mice lacking NPC2 mirror the symptoms seen in humans with NPC. Like humans, mice lacking NPC2 have been studied and also show great variability in disease onset and progression, depending on the genetic background. We have shown that NPC2 null mice on an FVB/N background show an increase in lifespan and decrease in disease progression compared to NPC2 null mice on the 129P2 background. Using standard histological techniques to assess loss of cerebellar purkinje cells and a behavioral test for motor coordination and learning, we plan to assess the effect of genetic modifiers of the onset and progression of neurodegeneration in NPC2 null mice.

P-49**INTESTINAL-SPECIFIC PPAR_γ DEFICIENCY ENHANCES TUMORIGENESIS IN APC^{MIN/+} MICE.**R Cormier¹, C McAlpine¹, Y Barak²¹ University of Minnesota Medical School, Duluth, MN, United States, ² Jackson Laboratory, Bar Harbor, ME, United States

Several studies have shown that *Apc*^{Min/+} mice treated with synthetic peroxisome proliferator-activated receptor γ (PPAR γ) ligands developed significantly more tumors in the colon. However, other studies employing different synthetic PPAR γ ligands have reported contradictory results in Min mice, in other *Apc*-deficient models and in chemically treated mouse models of colon cancer. In these latter studies, mice treated with PPAR γ agonists developed fewer tumors in both the small and large intestine. We have further tested the role of PPAR γ in murine intestinal tumorigenesis using *Apc*^{Min/+} mice carrying an intestinal-specific conditional knockout of PPAR γ (PPAR γ ^{Flox}; villin Cre). We observed that both heterozygous and homozygous intestinal-specific PPAR γ deficiency significantly enhanced Min tumorigenesis. The increase in tumor number occurred in both the small and large intestine but the most statistically significant effect was in the colon, where PPAR γ deficiency also modulated tumor incidence. We also found that gender significantly affected tumor multiplicity independent of PPAR γ genotype. Female Min mice developed more total tumors and more tumors in the small intestine but male Min mice developed more tumors in the colon. However, PPAR γ deficiency in the intestine enhanced tumorigenesis relative to PPAR γ -sufficient Min mice of each gender. Finally, we also examined the effect of germline haploinsufficiency for PPAR γ on the Min phenotype. Notably, while PPAR γ haploinsufficient females developed more Min tumors than PPAR γ ^{+/+} Min females, the Min phenotype of PPAR γ haploinsufficient males did not differ from that of PPAR γ ^{+/+} Min males. Overall our results support the role of PPAR γ as a tumor resistance factor in the mouse intestine and should prompt further studies of the PPAR γ -dependent and independent actions of synthetic PPAR γ ligands.

P-50**ENU-BASED MUTAGENESIS: IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF GPR33, MC3R AND MC4R RECEPTOR VARIANTS**

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INGENOTyping is a technology platform to generate gene-driven inbred mouse models based on a mutagenized parallel sperm and somatic DNA archive. **The archive comprises over 300.000 independent and evenly distributed gene-specific point mutations**, which translate into a 10-fold genome coverage on a gene-by-gene basis. This allows the recovery of an average of 10 independent alleles and respective mouse models per average-sized target gene. Simple two-step process of DNA archive analysis and *in vitro* fertilization (IVF) increases the speeds and reduces process risks when compared to other reverse genetics technologies. The specifics of the archive are detailed in Augustin *et al*, (Mammalian Genome, 2005).

To demonstrate that ENU mutagenesis delivers a high percentage of valuable mutations we screened a non-redundant subset of our DNA archive representing 9,367 DNA samples for mutations in the Gpr33, Mc3r, and Mc4r genes. 28 Mbp of coding sequence was analyzed and a total of 15 mutations (13 missense, 2 silent) were identified. Receptor variants for Mc3r (n = 3), Mc4r (n = 5) and Gpr33 variants (n = 5) were investigated by functional assays *in vitro*. **From our results we conclude that 12 out of the total of 13 missense mutations had significant effects on signal transduction properties** of the mutant alleles (Manuscript in preparation). In summary, the effect of ENU-induced missense mutation is much stronger than assumed and delivers a spectrum of gene function similar to those known from human diseases of complex genetic origin.

P-51**GEMIN2 PLAYS AN IMPORTANT ROLE FOR STABILIZATION OF THE SMN COMPLEX**

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The survival motor neurons (*SMN*) gene is responsible for the neurodegenerative disorder spinal muscular atrophy (SMA). *SMN* is a core component of a self-assembled multi-protein complex (the *SMN* complex) that contains *SMN* and Gemin2-7, and plays an essential role in the assembly of the spliceosomal small nuclear ribonucleoproteins (snRNPs). To gain insight into the molecular basis of SMA, we systematically explored association of each component within the *SMN* complex using the mammalian two-hybrid system. We found two novel interactions between Gemin2 and Gemin2 (self-interaction) and between Gemin2 and Gemin7, which were confirmed *in vitro* pull-down assay. The *in vitro* dissociation assay exhibited that self-interaction of the *SMN* protein and the *SMN*-Gemin7 interaction, both of which have been already known, became significantly stable in the presence of Gemin2. Thus, we concluded that the novel interactions play a role for the stabilization of the *SMN* complex.

P-52**ENU ARCHIVE OF DNA AND SPERM - A MUTATION IN EVERY MOUSE GENE**

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Parallel archives of frozen sperm and DNA have been established from F₁ ENU (N-ethyl-N-nitrosourea) mutagenised mice for the purpose of generating an allelic series in any mouse gene. Once a mutation is identified then mutant mice can be rederived easily by *in vitro* fertilisation (IVF) for phenotyping. As ENU induces point mutations it can be expected that the full range of functional changes in any gene might be uncovered – including amorphs (null), hypomorphs, hypermorphs and neomorphs.

We have now carried out a screen of 28 genes and demonstrated the applicability of this resource for recovering multiple alleles in all genes of the mouse genome. DHPLC has enabled us to identify 41 potential functional changes (missense and stop mutations) representing a frequency of 1 in 1.57 Mbp – this compares with the predicted rate of 1 in 2.38MB. For 31 alleles mutant mice have been recovered by IVF and all carried the predicted mutation. The archive consists of over 5,000 samples with a target of 10,000 in the near future; it is available to academics as a community resource and requests for DNA should be made to: gems@har.mrc.ac.uk.

ENU gene-driven screens are a powerful adjunct to mouse mutagenesis strategies. It can be expected that this resource along with others will be a powerful tool for developing multiple allelic series for each mouse gene as we address the challenge of systematic functional annotation of the mouse genome.

<http://www.mgu.har.mrc.ac.uk/facilities/dnaarchive/>

P-53**ABNORMAL PROCECESSING OF CAVEOLIN IN ALZHEIMER DISEASE MICE**

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Alzheimer's disease (AD) is characterized by progressive dementia and the accumulation of neuritic plaques in limbic and neocortical brain regions and tau deposition. Cholesterol is not only modulates amyloid beta synthesis but also interact between amyloid beta and neuronal membranes that may initiate a neurotoxic cascade. Caveolin, which binds with cholesterol, plays a prominent role in cellular cholesterol transport. High levels of cholesterol and caveolin in the caveolae-enriched fractions were found in AD patients compared with control. In the present study, we have used transgenic mice overexpressing mutated amyloid precursor protein (APP^{sw+V717F}) under the control of PrP gene promoter, and unique is that exhibits amyloid beta deposits by three month of age with dense-cored plaques and neuritic pathology. The Tau P301L transgenic line showed neurofibrillary tangles, astrocytic plaques, glial activation and tau filaments in cortex, hippocampus and other brain regions. Fibroblast cultures and brains from these mice were processed for caveolin expression. Western blot analysis of Caveolar markers, such as, Caveolin and Caveolin-1 showed significant increase in brain homogenates from 6 and 9 month old transgenic mice but no increase in young mice at 2 to 4 month of age compared with littermate control. There was significant increase in caveolin markers in fibroblast cultures established from APP^{sw+717} and Tau P301L lines compared with control. These results, taken together, suggest that caveolin expression is directly correlated with high Amyloid beta levels, Tau and amyloid deposits.

P-54**GLOMERULOCYSTIC KIDNEY DISEASE IN MICE WITH TARGETED INACTIVATION OF *WWTR1***

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WW domain containing transcription regulator 1 (*Wwtr1*) encodes a transcriptional co-activator with PDZ-binding motif known as TAZ that regulates the activity of transcription factors containing L/PPXY motifs. *Wwtr1* is highly expressed in the kidney and in other organs including liver, lung, heart and testis. To elucidate the physiological role of this gene we have inactivated *Wwtr1* in mice with *lacZ* knock-in vector using a homologous recombination based approach. Half of the homozygous mice died by the age of weaning, however, surviving offspring develop anemic and enlarged kidneys with corticomedullary cysts. Cyst formation begins at E16.5 and slowly spread throughout the kidney leading to end stage renal failure. Cysts are originating predominantly by the dilatation of Bowman's spaces and atrophy of glomerular tufts indicating that *Wwtr1*^{-/-} mice are manifesting phenotypes similar to glomerulocystic kidney disease (GCKD) in man and animal. Analysis of expression levels of GCKD associated genes (*Hnf1*□, *Tsc1*, *Odf1*, *Bicc1* and *Nphp1*) showed significantly decreased expression level of *Tsc1* and *Odf1* in homozygous embryonic kidney. Further, loss of cilia on epithelial cell lining of cysts and decreased expression levels of microtubule/intraflagellar transport (IFT) related genes (*Dctn5*, *Tg737*, *Odf1* and *Kif3a*) in the homozygous embryonic kidney strongly suggests that defective transcriptional co-activation of multiple cilia associated genes is the leading cause of the GCKD phenotype in *Wwtr1*^{-/-} mice. Thus, *Wwtr1* regulates a gene network essential for cilia integrity and kidney development and is a novel candidate gene for GCKD.

P-55**MOUSE MODELS: MUTANT PHENOTYPES AND HUMAN DISEASE**

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Mammalian model organisms are critical experimental surrogates for studying human biology and disease. The sophisticated genetic tools and sequenced genome of the mouse make it an exceptional model system for connecting knowledge from genome-to-phenotype-to-disease. The Mouse Genome Informatics Database (MGI, <http://www.informatics.jax.org>) supports biological knowledge building for the laboratory mouse by integrating and providing access to such data and thus forms a nexus for exploring and exploiting disease models.

As the initial step in supporting human disease querying, MGI is utilizing the Online Mendelian Inheritance in Man (OMIM) disease terms as a vocabulary base for annotations and searching. A robust system is being developed of human-friendly interfaces for querying and retrieval of disease and phenotype information, and the support of computational analyses of comparative phenotypes and diseases. Current annotations in MGI include over 1,180 mouse genotypes associated with human disease phenotypes, representing over 990 unique genes.

Examples will be provided illustrating the power of searching disease models in MGI and the ability to use these data in spanning the genome-to-phenotype-to-disease space.

Supported by NIH grant HG00330

P-56**THE MOUSE QUAKING PROTEIN (QKI) MAY AUTO-REGULATE ITS OWN mRNA**

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The *quaking* (*qk*) mouse mutant allele series displays varying phenotypes, ranging from abnormal vascular development causing embryonic lethality to the neurological phenotypes of dysmyelination, ataxia and epilepsy. The mutations have been localized to the *qkl* gene on proximal mouse chromosome 17, which encodes for a STAR (signal transduction and activation of RNA)-domain containing RNA-binding protein. The target consensus binding sequence for the QKI protein has recently been identified and we have found this sequence to be located in the 3'-untranslated region (UTR) of the *qkl* gene. This, along with our finding that when the nuclear isoform is not produced the two cytoplasmic isoforms are affected, suggests that the QKI protein may be auto-regulating its own mRNA through the use of the consensus binding site. Auto-regulation has been shown to occur in other RNA-binding proteins, including the Fragile X RNA binding protein (FMRP). Further analysis will confirm if QKI is auto-regulating its own RNA. If so, it suggests a new level of regulation of *qkl*. Alterations in this regulation could be causing the phenotypes seen in *qk* mutant mice and could have implications for other RNA-binding proteins.

P-57**ANALYSIS OF A NEW RECESSIVE MUTANT WITH ABNORMAL WALKING**

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The abnormal walking mutation arose spontaneously on the *Il6* deficient mouse strain. Affected mice raised their hind legs much higher than normal mice, and exhibited awkward walking. In addition affected mice showed smaller eyes with retinal malformation and degeneration. While affected females could breed, affected males were sterile, probably due to difficulties in mating behavior. The results of test crosses clearly indicated that this mutation was inherited as an autosomal recessive mutation, and confirmed to be independent from the *Il6* deficient condition. Haplotype analysis of F_2 mice indicated that the responsible gene was located at the 6.48 Mb region between *D19Mit61* and *D19Mit111* on mouse chromosome 19. The overall behavioral and histopathological phenotypes of the affected mice were quite similar to those described for the hugger (*hug*) and the *Rorb* deficient mice in which the responsible genes are located on chromosome 19. The causative gene of the former allele has not been defined yet. In order to evaluate *Rorb* as a candidate gene of the mutant, cDNAs of *Rorb* were isolated from the homozygous mutants and the normal litter mates, and sequenced to determine the molecular lesion of this mutation. It was found that the mutant had a point mutation in the coding region, resulting in a single amino acid substitution. This amino acid residue is highly conserved among several species, suggesting that the mutation in this conserved sequence may be responsible for the mutant phenotype.

P-58**A SET OF HIGHLY INFORMATIVE RAT SIMPLE SEQUENCE LENGTH POLYMORPHISM (SSLP) MARKERS AND GENETICALLY DEFINED RAT STRAINS IN THE NATIONAL BIO RESOURCE PROJECT FOR THE RAT (NBRP-RAT)**

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The National Bio Resource Project for the Rat in Japan (NBRP-Rat) is collecting, preserving and distributing many rat strains, including spontaneous mutant, transgenic, congenic, and recombinant inbred strains, in Japan as well as all over the world. To evaluate their value as models of human diseases, we are characterizing them with a total of 109 comprehensive phenotypic parameters: clinical measurements, internal anatomy, metabolic parameters, and behavioral tests, as part of the Rat Phenome Project. Here, we report a set of 357 simple sequence length polymorphism (SSLP) markers and 122 rat strains which were genotyped by the marker set. The SSLP markers were selected according to their distribution patterns throughout the whole rat genome with an average spacing of 7.59 Mb. The average number of informative markers between all possible pairs of strains was 260 (73% of 357 markers), representing their high polymorphisms. From the genetic profile, we constructed a family tree of the rat strains to clarify their genetic background. A set of these highly informative SSLP markers as well as the genetically defined rat strains are useful to design experiments for quantitative trait loci analysis and to choose strategies for developing new genetic resources. These data and resources are freely available for any interested researcher worldwide at the NBRP-Rat (www.anim.med.kyoto-u.ac.jp/nbr).

P-59**BCL11B IS A HAPLO-INSUFFICIENT TUMOR SUPPRESSOR GENE CONTROLLING THE S PHASE CHECKPOINT**

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Bcl11b/Rit1 is a tumor suppressor gene that was isolated by allelic loss mapping and positional cloning using mouse thymic lymphomas. Bcl11b gene encodes zinc-finger proteins and plays a key role in abT-cell development whereas its paralog Bcl11a/Evi9 functions for B-cell differentiation. Chromosomal rearrangements within this gene are found in human T-cell leukemias, but how this gene disruption contributes to leukemogenesis is not known. Here we show that Bcl11b is a haplo-insufficient tumor suppressor gene, by demonstrating that Bcl11b^{+/-}-p53^{+/-} mice have greater susceptibility to lymphomas than Bcl11b^{+/+}-p53^{+/-} mice, but most lymphomas retain and express the wild-type Bcl11b allele. This is further supported by that Bcl11b^{+/-} mouse embryos exhibit impairment in the development and survival of thymocytes. Finally, we show the function of Bcl11b proteins using RNAi technology that was applied to Jurkat human T-cell and FRSK skin keratinocyte cell lines. Bcl11b down-regulation decreases cell growth by increased apoptosis and lowers the number of S phase cells. Interestingly, knock-down cells reduced the phosphorylation level of cell-cycle checkpoint kinase 1, Chk1, that controls the S phase checkpoint, maintaining the genome stability. Thus, Bcl11b is a new member in the ATR-Chk1 pathway that controls activation of Chk1 through the phosphorylation.

P-60**NOVEL MOUSE MODELS OF DIABETES MELLITUS GENERATED BY THE RIKEN ENU MUTAGENESIS PROJECT**

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One specific aim of our project is to generate diabetic models that will enable us to clarify the functions of genes and the pathogenic mechanisms of human diabetes.

We mutagenized C57BL/J (B6) males and mated them with DBA/2J (D2) females. In this D2xB6-based mutagenesis protocol, we screened approximately 12,000 animals for early-onset dominant traits using a clinical biochemical test and thereby identified and reported 12 glucokinase (*Gck*) mutants that were highly useful models of human MODY2 and PNDM. The analyses of the heterozygous mutants showed that all these mutants displayed similar phenotypes to human MODY2 patients, while their various point mutations affected differently in *Gck* mRNA and protein expressions. We will present the variation of phenotypes detected in the homozygotes and compound heterozygotes of several *Gck* mutants. We have been investigating other diabetic mutants that their phenotypes are distinct from the *Gck* mutant-like phenotypes. Results from phenotypic and/or genetic analyses for these new mutants will be also presented.

To expand a diabetic spectrum of the phenotype-driven mutagenesis, we have initiated new screening protocols. First, to assess the effect of genetic background, we have used C3H/HeJ females as mating partners for the mutagenized B6 males. Second, to generate the metabolic syndrome-related diabetic models, we have initiated recessive screenings using B6, and high-fat-diet feeding to some part of pedigrees in the recessive screening. The review and progress of these new screenings will be presented.

P-61**THE GERMAN MOUSE CLINIC – AN OPEN ACCESS PLATFORM FOR SYSTEMATIC, COMPREHENSIVE AND STANDARDIZED PHENOTYPING OF MUTANT MOUSE LINES (MMLS)**

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We have established a worldwide unique phenotyping center, the German Mouse Clinic (GMC), as part of the German National Genome Research Network (NGFN) at the National Research Center for Environment and Health (GSF) in Munich (www.mouseclinic.de). This phenotyping platform is designed to provide the scientific community with systematic, standardized and comprehensive phenotyping of mutant mouse lines (MMLs) on various genetic backgrounds and generated by different methods (transgenic and knockout mice, mutants from ENU or other mutagenesis screens and gene-trap approaches). The integration of laboratories and mouse rooms in one building ensures optimal interdisciplinary communication and close interaction between experts from different fields, institutions and backgrounds.

Mouse mutants are examined in a comprehensive primary screen by using state-of-the-art morphological analyses and functional tests covering the areas bone and cartilage, behavior, neurology, eye, clinical chemistry, immunology, allergy, nociception, cardiology, molecular phenotyping, lung function, energy metabolism, steroid metabolism, and pathology. The exceptional advantages of our primary screen are the standardized measurements of approximately 240 quantitative and qualitative parameters of each individual mouse. This detailed phenotypic analysis is offered on the level of scientific collaborations.

To date, the phenotyping in the primary screen for 40 MMLs from institutes all over world has been finished. In nearly all of these mutants, we were able to detect altered parameters in the mutants compared to their controls. Secondary screens for most of the mutants, which showed new phenotypes are already in progress.

This work is supported by the NGFN.

P-62**PHENOTYPING THE MOUSE – EMPRESS, A EUROPEAN MOUSE PHENOTYPING RESOURCE FOR STANDARDISED SCREENS**S Brown, [H Gates](#)

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With the completion of the mouse genome sequence, one of the key goals for functional genomics is the creation of a series of mutant alleles for every mammalian gene. Large-scale mutagenesis efforts are underway using both gene-driven and phenotype-driven approaches to generate this resource of mouse mutants. An even greater challenge will be the determination of the phenotypic outcomes of each mutation. A vital element of this endeavour will be to develop standardised phenotyping platforms that allow for reproducibility of test outcome over both time and place. The EUMORPHIA Program is a consortium of 18 research institutes from across Europe working on establishing new methods in phenotyping with a focus on improving and standardising phenotyping platforms for the mouse. Eumorphia has developed a new robust primary screening platform, EMPReSS - European Mouse Phenotyping Resource for Standardised Screens. This primary screen incorporates over 100 new SOPs, each validated on a cohort of inbred strains across a number of laboratories. EMPReSS covers all of the major body systems, as well as generic approaches in pathology and gene expression. We have developed a database and web-based resource for the visualisation, searching and downloading of the EMPReSS SOPs and other documents that constitute EMPReSS. The availability of new standardised screens and associated informatics structures and tools will be a vital underpinning for a systematic and rational functional annotation of the mouse genome and a necessary step in improving the mouse as a tool in the biological studies and drug discovery.

P-63**SEIZURES IN STARGAZER AND LETHARGIC DOUBLE MUTANT COMBINATIONS: MOUSE MODELS FOR HUMAN ABSENCE EPILEPSY**[V A Letts](#), W N Frankel

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The stargazer mouse has a mutation in the *Cacng2* gene encoding the stargazin or gamma2 protein. In addition to stargazer, we have two further allelic mutants; waggler and stargazer 3J. Interestingly, the phenotypic of each mutation is subtly different. Stargazer is the most profoundly affected, with an ataxic gait, prolonged absence seizure episodes and a distinctive head lifting activity. In contrast, stargazer 3J, the mildest of this allelic series, displays an ataxic gait but no absence seizure activity.

Stargazin (gamma2) has been associated with two functions in the brain; voltage-dependent calcium channel (VDCC) activity and AMPA receptor localization. Is the disruption of both processes in the cortex and thalamus contributing to seizures, or does one pathway predominate in controlling seizure activity in these critical regions? To attempt to address this question, we have constructed double mutants between stargazer 3J and lethargic 2J mice. Lethargic 2J has a mutation in the VDCC beta4 subunit gene, *Cacnb4*. This mouse also has absence seizures, indicating that VDCC mutations can alone cause absence seizures. We observe increased seizure activity in the double homozygous mutants, and we are pursuing this result further with heterozygous;homozygous combinations to discover more about the functional interaction between these two proteins.

P-64

GENERATION OF KO-LIKE ANIMALS BY INDUCTION OF AUTOIMMUNE RESPONSE AGAINST THE TARGET PROTEIN

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We hereby describe a new technology we developed for the neutralization of secreted and receptor proteins via the direct in vivo induction of a specific antibody response. The presentation will show examples we obtained in mice against different secreted proteins.

The developed strategy is based on specific mutation in the coding sequence of the target protein, specifically to induce an auto-immune response against the unmodified target following vaccination of animals with recombinant adenoviruses encoding the different mutants.

Following vaccination, mice are followed by immunological assays to demonstrate the induction of a specific antibody response against the endogenous unmodified target. The neutralization of the biological functions of the target protein is demonstrated by in vitro assays or by characterization of a specific phenotype in vivo.

In our experiments, depending on the proteins targeted the KO-like phenotypes are observed one to two months following vaccination.

The comparison of the obtained phenotypes showed similarity to the ones described for the corresponding knocked-down mice generated by the classical homologous recombination technique.

The advantages of the NOKAD technology will be discussed by comparison with genetic knock-out by homologous recombination, siRNA, embryonic cloning, or chemical mutagenesis.

Among advantages of the NOKAD technology, we can list, fast obtaining of Functional-KO animals, 6 months for the first derived phenotype, two months when secondary experiments are needed; reduced costs of animal breeding; functional KO for all mammalian species: from mice to primates in all backgrounds; developmental and post-developmental functions can be studied; multiple functional-KO; phenotype reversion by immunosuppression.

P-65

FRIEDREICH ATAXIA NEUROLOGICAL MOUSE MODEL DEMONSTRATES CASPASE-INDEPENDENT CEREBELLAR GRANULE CELL DEATH WITH AUTOPHAGY AND CATHEPSINS INDUCTION

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Friedreich ataxia (FRDA), the most common recessive ataxia, is a progressive neurodegenerative disease associating degeneration of the large sensory neurons and spinocerebellar tracts, and cardiomyopathy. It is caused by severely reduced rate of frataxin, a mitochondrial protein involved in iron-sulfur cluster biosynthesis. Through a spatio-temporally controlled conditional gene targeting approach, we have generated two neurological mouse models which develop progressive mixed cerebellum and sensory ataxia. One model develops a progressive loss of the cerebellar granule cell (CGC) in addition to the degeneration of the sensory neurons of the dorsal root ganglia (DRG), spinocerebellar tract and posterior column. We previously identified an autophagic process as the causative pathological mechanism in the DRG. We now report the characterization of the CGC death. Ultrastructural studies show morphological evidences that cannot be classified into a single cell-death category (necrosis, autophagy, apoptosis). Furthermore, expression studies demonstrate a combined activation of the cathepsins and autophagic markers, with no caspase activation. Morphological evidences suggest the involvement of AIF, a key factor in caspase-independent apoptosis. We are thus currently exploring the subcellular localisation of AIF to determine whether it is implicated. Finally, as there are increasing evidences of the involvement of cell cycle reactivation in the process of neurodegeneration, we are investigating the expression of different cell cycle proteins. Our preliminary results suggest that prior to the massive cell loss, cell cycle specific markers are expressed. In conclusion, we have generated mice which represent excellent models for FRDA, and which enable us to study the different pathways leading to neurodegeneration.

P-66**INDUCTION OF HEAT SHOCK PROTEINS BY HEAT AND NOISE STRESS IN THE NORMAL, AGED, AND *HSF1* KNOCKOUT MOUSE COCHLEA**

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Hearing loss is a major health problem for both young and old. Excessive noise can cause either transient or permanent hearing loss. Our research focuses on protective mechanisms regulated by heat shock transcription factor 1 (HSF1) in the cochlea. Many stresses activate HSF1, leading to induction of heat shock proteins (Hsps). To understand how HSF1 protects the cochlea from noise stress, we examined induction of Hsps in wild-type, *Hsf1*^{+/-}heterozygotes, and *hsf1*^{-/-}mice, following both heat shock and noise stress, using quantitative RT-PCR with gene-specific TaqMan probes. Genes encoding heat shock proteins Hsp25, Hsp47, Hsp70 (*Hsp70.1*, *Hsp70.3*), Hsp84, Hsp86, and Hsp110 were induced following heat stress in heterozygotes, but not in *Hsf1*^{-/-} null mice. In wild-type CBA mice, induction of *Hsp25*, *Hsp70.1*, and *Hsp70.3* by heat stress decreased between 12 – 22 months, suggesting that this decrease might contribute to presbycusis. The induction of Hsps by noise overstimulation was examined following exposure to BBN (2-20 kHz) presented for 2 hr at increasing noise intensities. Hsp70.3 mRNA showed 4.5-fold, 6.5-fold, and 19-fold induction at 98, 106, and 120 dB SPL, respectively. Maximum induction for most Hsp genes occurred 4 hr following the noise exposure. Noise stress induced Hsp70.3 about 3-fold in *Hsf1* KO mice, suggesting that other factors induced Hsp70 following noise over stimulation in the cochlea. To examine the effect of noise stress in the *Hsf1*^{-/-} mouse cochlea with age. For aging studies we created a new mouse model carrying the resistant allele of the *Ahl* locus on Chr 10 from CBA.

P-67**AN EXTENDED QTL ANALYSIS FOR SKIN TUMOR SUSCEPTIBILITY IN DOMINANT RESISTANT BACKCROSSES**

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A great variety of cancer susceptibility among mouse strains reveals genetic background differences which contribute cancer development. The two stage chemical skin carcinogenesis model [induced by dimethylbenzanthracene (DMBA)/ 12-O-tetradecanoylphorbol-13-acetate (TPA)] has been analyzed extensively and results from a dominant resistant *mus spretus* cross experiment indicated that a responsible gene for mouse skin tumor susceptibility loci 13 (*Skts13*) was also involved in human cancer risk. We have extended this study searching the other dominant resistant strain and found a two genetically diverged inbred cross, FVB/N and PWK. The susceptible FVB/N developed more than 30 papillomas after the treatment, while resistant PWK hardly developed papillomas. (FVBxPWK)F1 mice also developed few tumors after the treatment, indicating that the PWK is a dominant resistant strain. To map *Skts*, we performed linkage analysis of 178 F2 progenies in a (FVBxPWK)F1 x FVB backcross, so far genotyped 96 microsatellite markers with an average intermarker distance of 30 cM. QTX version 0.30 was used for linkage. A significant linkage was found on chromosome 4 with LOD score 7.2 and suggestive linkages were on chromosomes 1, 11 and 14. In addition, we analyzed 97 F2 progenies in a reciprocal FVB x (FVBxPWK)F1 backcross. Interestingly, linkages on chromosome 4 and 1 were disappeared in theses crosses. This result suggests the responsible genetic mechanisms at the *Skts* loci, such as an imprinting, maternal regulation or Y-linked modification. We will also discuss genetic basis of skin tumor susceptibility comparing data from the previous QTL studies.

P-68**MOUSE MODEL OF NEPHROGENIC DIABETES INSIPIDUS**

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In mammals, the tight hormonal regulation of water homeostasis is mediated by the aquaporin water channel (AQP2) of the collecting duct (CD). Vasopressin induces redistribution of AQP2 from intracellular vesicles to the apical membrane of CD principal cells, accompanied by increased water permeability. Mutations of *AQP2* gene cause recessive and dominant nephrogenic diabetes insipidus (NDI), a disease in which the kidney is unable to concentrate urine in response to vasopressin. In mouse complete inactivation of this gene resulted in neonatal lethality. In this study, we generated mice with the distal C-terminal tail of the *Aqp2* deleted (*Aqp2*^{Δ231}), including the protein kinase A phosphorylation site (Ser-256), while still retaining the putative apical targeting signal (221-230) at the C-terminal. Homozygotes are viable to adulthood, with reduced urine concentrating ability: increased urine output (5-10 times more than control mice) and decreased urine osmolality (193 ± 17 in *Aqp2*^{Δ231/Δ231} vs 1,173 ± 130 mOsm in control mice), and increased daily water consumption. While desmopressin can increase urine osmolality in wild type mice, it has no effect on *Aqp2*^{Δ231/Δ231} mice. Kidneys from affected mice showed CD and pelvis dilatation and papillary atrophy due to polyuria. Immunohistological analysis using antibody against the C-terminal region of the protein could not detect any channels in the affected kidney, while CD-specific staining was obtained in wild type kidney. Thus we have generated a mouse model of NDI and it should be useful in studying the physiology and potential therapy of this disease.

P-69**THREE MOUSE MODELS FOR HUMAN HYPOPHOSPHATEMIC RICKETS GENERATED BY A LARGE SCALE N-ETHYL-N-NITROSOUREA MUTAGENESIS PROJECT**

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Three mouse mutants, showing increased serum alkaline phosphatase activity and decreased inorganic phosphate level, were identified in the course of blood test screening of mouse *N*-ethyl-*N*-nitrosourea mutagenesis project in RIKEN, Japan. These abnormalities suggested failure in the function of bone metabolism. X-ray images of knee joints of mutants showed disorder of growthplate and widening of the metaphyseal ends of the tibia. These were the same features as those of human rickets patients. By statistical analysis of clinical biochemical data, small but significant difference among three mutant lines was observed, suggesting there is a different phenotype severity among mutants. Gene mapping data indicated that responsible regions of three mutants were located on X-chromosome and the *Phex* gene was considered as a candidate gene because human *PHEX* is a responsible gene for X-linked hypophosphatemic rickets (XLH). As expected, point mutations were identified in the *Phex* gene in all three mutants. Two mutants carried nonsense mutations and one had a mutation for splicing abnormality. By RT-PCR analysis, we observed mRNA degradation by nonsense mediated mRNA decay pathway. Also, exon skipping was observed by sequencing the amplified products. mRNA decay blocks the production of mutant protein, while exon skipping causes the production of truncated protein which may have full or partial activity if reading frame is conserved. Variation of the combination of two pathways may correspond the variation of phenotype severity.

P-70**A NEW MUTANT MOUSE (SLW) WITH ACHONDROPLASIA CAUSED BY A MUTATION IN CNP RECEPTOR GENE**

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Short-limbed dwarfism (SLW) is a new mutant mouse strain established in Okayama University showing a dwarf phenotype characterized by short body, limbs and tail and domed shape skull. The dwarf phenotype appears at seven days after birth and a significant part of the affected mice dies before weaning. Histological examination showed disturbed chondrogenesis during endochondral ossification. The phenotype inherited as an autosomal single recessive mode and the gene for this phenotype has been termed *slw*. In the present study, we identified the causative gene for the SLW mouse. Linkage analysis showed that *slw* was localized on a 10 cM region of the mouse chromosome 4 between *D4Mit172* and *D4Mit139*. The localization of *slw* on mouse chromosome 4 was same as that of the locus for CN mutant mouse which shows a dwarf phenotype resembling to that of SLW mouse, suggesting that *slw* and *cn* is alleles of the same locus. We have previously revealed that the *Npr2* gene, which encodes a receptor for C-type natriuretic peptide (CNP) possessing guanylyl cyclase activity, is the causative gene for the CN mouse. We, therefore, determined the nucleotide sequence of the *Npr2* gene in the SLW mouse and found a deletion of 7 bases in exon 8. The chondrocytes of the mutant mouse showed no increased guanylyl cyclase activity upon CNP stimulation. We concluded that the deletion in *Npr2* gene is responsible for the dwarf phenotype of the SLW mouse.

P-71**PHENOTYPING THE MUTANT MOUSE ORAL CAVITY**

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Tooth development is under strict genetic control and involves continuous progressive inductive interactions between defined sites of the oral ectoderm and ectomesenchymal cells derived from the cephalic neural crest. Disruptions of the involved signalling pathways and associated transcription factors lead to dental anomalies. These defects are seen in Human isolated dental genetic diseases and in numerous syndromes. Of the over 5000 genetic syndromes known more than 700 have dental/oral/craniofacial anomalies and over 250 have associated clefting. The mouse dentition is a powerful and useful model to study the genesis of Human dental anomalies. A number of transgenic mice generated so far display dental defects that mimic the pathology encountered in Human. It is important to analyse precisely and systematically the oral phenotype within the general phenotypical screen for mutant mouse models, implemented at the Mouse Clinical Institute and involved in the Eumorphia research program. The aims of this systematic oral phenotyping work are to define, validate and implement a standard screening protocol to monitor tooth development and homeostasis and to detect orodental defects. Examples of analysis of these transgenic mice are given: *rsk1,2,3* triple knockout (ribosomal S6 kinase family members) belonging to a group of X chromosomal genes in which defects cause unspecific mental retardation in human like Coffin-Lowry syndrome.

This phenotyping Program will be useful for the scientific community and especially for the scientists grouped in the European COST Action B23 Oral Facial Development.

P-72**ENU-INDUCED MOUSE MUTANT WITH BEHAVIORAL ABNORMALITIES RELEVANT TO ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD)**

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Recent studies have demonstrated that psychiatric diseases such as schizophrenia and bipolar disorder have some genetic basis. In human populations, however, identification of genes underlying psychiatric disorders is still difficult because of polygenic inheritance and gene-environment interactions. Genetic studies using the mouse as a model have great advantage for identification of molecular pathways of behavior. In order to establish animal models of human psychiatric disorders, we have been carrying out ENU-mutagenesis screening for dominant psychiatry-relevant behavioral abnormalities. One of our targets is hyperactivity in the open-field and home-cage, because hyperactivity is a core symptom of attention deficit hyperactivity disorder (ADHD). We have screened about 2,500 G1 animals and obtained 7 hyperactive mutants. As a secondary screening for ADHD relevant behavioral abnormalities, we examined the effect of methylphenidate in three hyperactive mutants; M-73, M-174, and M-914. Methylphenidate (Ritalin) is a psychostimulant that increases extracellular dopamine concentration and enhances the activity level in normal subjects, but it suppresses hyperactivity in ADHD paradoxically. By methylphenidate administration (30mg/kg), M-73 and M-914 increased locomotor activity in the open-field, while M-174 did not change the activity. M-174 also showed passive avoidance deficit and reduced level of explorative behavior to a novel object. It suggested that M-174 has impulsiveness (impairment of behavioral inhibition) and attention deficit. The causative gene of M-174 was mapped to a 1.5Mbp region on proximal chromosome 2, and we are sequencing several candidate genes. M-174 would provide an insight to elucidate the pathogenesis of ADHD .

P-73**BEHAVIORAL ANOMALY OF AN ENU-INDUCED MUTANT LINE M-200 WAS AFFECTED BY THE GENETIC BACKGROUND**

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M-200 is a dominant mutant line that was isolated in the RIKEN ENU-mutagenesis project. The founder mouse M1_200 was originally detected as a learning deficit mutant in passive avoidance test. In the later analysis, M1_200 showed not only learning deficit but also reduction of body size, convulsive seizure, and immobility. The convulsive seizure and immobility start suddenly with vocalization, ataxic gait, lowered posture, and slow movement. These symptoms start 30-60 minutes after mice were transferred to new home cage. To identify the causative gene, we carried out linkage analysis for the phenotype of learning deficit or immobility. As a result, the causative gene was mapped to a distal region of 4.8cM interval between *D4Mit332* and *D4Mit249*.

Subsequently, we have introduced the M-200 mutation into backgrounds of two mouse strains, C57BL/6J and C3H/HeJ, by phenotype-assisted and genotype-assisted backcrosses respectively. In these crosses, it appeared that about 50% of N8 progeny generated from backcrosses to C57BL/6J strain showed either convulsive seizure or transient immobility, or both. By contrast, none of N6 progeny generated from backcrosses to C3H/HeJ strain showed these symptoms. F1 progeny heterozygous for the M-200 mutation, which was generated from cross of the heterozygous C3H/HeJ-M-200(N6) and C57BL/6J, showed both of convulsive seizure and transient immobility. Thus, the result of this study suggested that genetic background markedly affects phenotypes of M-200.

P-74**MOUSE MUTANTS OF IMMUNE AND ALLERGIC DISEASE: RECENT RESULT OF THE RIKEN RCAI ENU MUTAGENESIS PROJECT**

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In RIKEN RCAI ENU mutagenesis project, to establish mouse models for immune and allergic disease, we screen for various phenotype in the mutated mice. We injected ENU into C57BL/6 male and obtained the next generation male mice each as the independent founders, and obtained the third next generation to produce the recessive mutant pool of each pedigree. Since we produced every mutant generation by in vitro fertilization and embryo transfer (IVF-ET), we could easily obtain a large number of mice of each pedigree at the same developmental stage under the collaborative study in the institute.

More than 48 mice per pedigree are bled at 12 weeks of age, and screened morphological and behavioral anomalies, hematological alterations and immunological defects. These mice are re-checked at 16 weeks of age. Mice are screened as a same at 12 weeks of age, as well as autopsied. Lymphoid organs such as thymus, lymph node, spleen and peritoneal exudate cells are harvested and are analyzed for surface marker on their cells, and histological analysis are performed.

To date, we obtained 31 pedigrees, 1821 G3 progeny and screened all mice for visible mutations. Furthermore 1325 mice (650 female and 675 male) in this ENU mutant mice pool are investigated for blood-based parameters in the first year of our ENU mutagenesis project. As a result, we have isolated 61 mutant lines such as allergy symptoms (3 pairs), increased number of white blood cells (10 pairs) and abnormal amount of serum immunoglobulin (38 pairs). These mice are under heredity confirmation.

And now, we have set up a new sensitized screen using the ENU G3 mice in order to identify enhancing or suppressing factor of pollen induced conjunctivitis, which lots of Japanese afflict with.

Recent result of non-sensitized and sensitized ENU mutagenesis screen will be shown in this presentation.

P-75**THE ENU-INDUCED MUTATION *DILP2* CAUSES LOSS-OF-FUNCTION OF THE ESSENTIAL X-LINKED ACTIN-BINDING PROTEIN FILAMIN A**S H Cross¹, J E Morgan¹, A W Hart¹, K West¹, L McKie¹, J E Schneider², S Bhattacharya², I J Jackson¹

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ENU mutagenesis is a powerful tool for revealing gene function. We have produced a collection of 25 ENU-induced mutants with a variety of eye defects. One, *Dilp2*, is an X-linked male lethal that causes mild pupil defects in carrier females. High-throughput magnetic resonance imaging of embryos revealed that *Dilp2* males have a common arterial trunk, a condition that accounts for 1% of all congenital heart defects in humans. In addition, they have mid-line abnormalities manifesting as palate defects and a failure of the sternum to fuse. We mapped *Dilp2* to a small interval and found a nucleotide substitution that generates a stop codon in the filamin A (*Flna*) gene. *Flna* encodes an actin-binding protein that forms an important signalling scaffold in the cell. In humans, heterozygous loss-of-function of *FLNA* causes periventricular nodular heterotopia in females and is lethal in hemizygous males whilst missense mutations underlie a spectrum of disorders affecting both males and females that feature skeletal dysplasia accompanied by a variety of other developmental abnormalities. *Flna* is undetectable in *Dilp2* mutant cells implying that the predicted truncated protein is either not produced or is unstable. Therefore the phenotype we see in hemizygous mutant males is due to loss-of-function. *Dilp2* provides a model for the study of this important human disease gene. The discovery of *Dilp2* in an ENU screen is particularly notable given the difficulties associated with generating mouse models of X-linked male lethal genes by a conventional gene knock-out approach.

P-76

FIMRE: FEDERATION OF INTERNATIONAL MOUSE RESOURCES

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Nineteen major mouse resource centers from around the world have established an international federation to collaborate to insure the continued availability of existing mouse resources and cope with the onslaught of new mutant ES cells and strains being created. The purpose of the organization is to coordinate repositories to share more effectively archiving and distributing strains of mice as cryopreserved embryos and gametes, ES cell lines, and live breeding stock to the research community. Specific goals are to coordinate archiving valuable genetically defined mice and ES cell lines being created worldwide, meet research demand for these genetically defined mice and ES cell lines, establish consistent, highest quality animal health standards in all resource centers, provide genetic verification and quality control for genetic background and mutations, provide resource training to enhance user ability to utilize cryopreserved resources. The current members of FIMRe are nine centers in North America, seven centers in Europe, two in Japan and one in Australia.

P-77

T-CELL LYMPHOMAS IN MURINE *IN VITRO* AND *IN VIVO* MODELS SHOW TRANSLOCATIONS INVOLVING CHROMOSOMAL BAND 15D3 OR TRISOMY 15

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Introduction: Activation of the oncogene *C-MYC* has been described in various diseases, e.g. in Burkitt's lymphoma, and can result from different mechanisms, e.g. translocations involving the *C-MYC* locus on human chromosome 8q24 or the murine chromosome 15D3 or from an increase in the gene dosage. We cytogenetically analyzed *N-RAS* transgenic mice with and without methyltransferase Suv39h1-deficiency and a cell line resembling the human anaplastic large cell lymphoma (ALCL). These models showed different alterations of chromosome 15.

Methods: Chromosome spreads were prepared from T-cells according to standard protocols. Spectral karyotyping (SKY) was carried out according to the manufacturer's instructions (Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel). For fluorescence *in situ* hybridization (FISH), a probe hybridizing to the *c-myc* locus on 15D3 was generated from BAC clone RP24-488H15.

Results: 3 of 4 clones from *N-RAS* transgenic mice with Suv39h1-deficiency and both clones from *N-RAS* transgenic mice without Suv39h1-deficiency showed translocations with breakpoints in 15D3. Different regions on chromosomes 6, 11, and 14 were involved as translocation partners. Thus, the methyltransferase deficiency does not seem to result in an enhanced chromosomal instability. Trisomy 15 was found in all 20 metaphases of the ALCL cell line.

Conclusion: These numerical and structural alterations of murine chromosome 15 may induce an activation of the *C-MYC* gene by different mechanisms.

P-78**THE EUROPEAN MOUSE MUTANT ARCHIVE (EMMA)**

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The EMMA (European Mouse Mutant Archive) was established as part of a worldwide network of repositories for retaining mutant strains essential for biomedical and medical research. Besides cryopreservation of mouse mutant lines in form of embryos or sperm, the distribution of these lines to qualified investigators is the main focus. EMMA maintained lines are supplied as a service to the research community at large and solely for research purposes. EMMA strains are available to academic institutions from all around the world. Applications for depositing and requesting mutant strains can be submitted through the EMMA website www.emmanet.org.

In order to make the transfer of biological information more effective, appropriate databases will be further developed in the framework of the EMMA and in collaboration with existing databases e.g. the IMSR (International Mouse Strain Resource). An essential role of the EMMA project is to foster the virtual coupling of the EMMA stock centers and related information systems through the establishment and maintenance of a dedicated resource database (EMMA-RDB). This is implemented as a fully integrated web-based database and serves as an interface to the scientific community.

EMMA as part of the global resource network for mutant mouse lines (FIMRE, the International Federation of Mouse Resources) actively cooperates with other leading repositories like the MMRRRC in the US and the Riken Labs from Japan. EMMA is supported by primary public research Institutions of the participating countries and by the European Commission

P-79**GENETIC DETERMINANTS FOR SUSCEPTIBILITY GENE UNDERLYING AGE-RELATED HEARING LOSS USING CONSONOMIC C57BL/6J.MSM STRAINS**

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Age-related hearing loss (AHL) is one of the most common chronic health problems of elderly individuals. One approach for unraveling the genetic basis of AHL is to use mouse models, and the C57BL/6J (B6) mouse has long been studied as a model of AHL. Here we show analysis of AHL susceptibility using B6.MSM consomic mice substituting a single *molossinus*-chromosome on the B6 genetic background.

1. *Ahl3*, a third locus on mouse chromosome 17 affecting AHL and noise-induced hearing loss (NIHL): The auditory brainstem response (ABR) of B6.MSM consomic mice at various ages was examined. One particular strain, B6-Chr17^{MSM} showed a prominent resistance, still having good hearing at 18 months of age. Subsequent mapping showed a significant association of ABR thresholds with a locus, designated as *Ahl3*, in the vicinity of *D17Mit119*. We also show that *Ahl3* affects NIHL, by showing that B6 mice exhibit permanent threshold shift (PTS) after one hour exposure to 100 dB noise while B6-Chr17^{MSM} do not.

2. Genetic interaction between *Cdh23* and *Sans* in the AHL susceptibility: Homozygous Jackson shaker (*js*) mice exhibit congenital deafness due to the mutation of a novel gene encoding a scaffold protein, SANS. Interestingly, we found that heterozygotes are haploinsufficient and exhibit susceptibility to a progressive and early-onset hearing loss. To look for other genes influencing this AHL susceptibility, we examined B6.MSM consomic mice of the *js/+* genotype. A significant linkage was provided with the *Cdh23* (*Ahl1*) locus, from the analysis of *js/+* B6-Chr10^{MSM}.

P-80**PHENOTYPIC ANALYSIS OF A MONOSOMIC MODEL FOR THE TELOMERIC REGION *HRMT1L1-COL6A1* ASSOCIATED TO HUMAN CHROMOSOME 21**

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Dosage imbalance of human chromosome 21 (HSA21) genes leads to physiological and morphological abnormalities in numerous organs of affected patients. Whereas trisomy 21 leads to Down syndrome, monosomy 21 (M21) is rarely viable and is associated to a very complex and variable phenotype. Genetic analyses of this pathology are restricted to rare partial M21 patients. The use of animal models such as the mouse is therefore necessary to determine the relationship between phenotype and genotype for this pathology.

HSA21 homologous regions are localized on mouse chromosomes 10, 16 and 17. The homologous region on chromosome 10 corresponds to a gene-rich segment corresponding to the telomeric part of HSA21 for which no mouse model exists. We therefore developed a new monosomic model for the *Hrmt111-Col6a1* region that contains 14 genes and spans 0,7Mb.

We created a deletion of the *Hrmt111-Col6a1* region using two chromosomal engineering approaches: the *Hprt* system (Zheng et al., N.A.R. 1999, 27, 2354-2360) and the TAMERE strategy (Hérault et al., Nat Genet, 1998, 381-384).

A mouse model of monosomy for the *Hrmt111-Col6a1* region was generated from the obtained deletion. During this meeting, we will present the characterisation of the mutant mice using a panel of phenotypic assays that unravel the presence of key genes with dosage effect.

P-81**PLASMINOGEN/STREPTOKINASE INTERACTION IS A CRITICAL HOST PATHOGENICITY FACTOR FOR GROUP A STREPTOCOCCAL INFECTION**

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Group A streptococci (GAS), a common human pathogen, secrete streptokinase (SK), which activates the host's plasminogen (PLG). SK is highly specific for human PLG, exhibiting little or no activity against other mammalian species. We generated a "humanized" transgenic mouse expressing human PLG under control of the mouse albumin gene within a Bacteria Artificial Chromosome (BAC) transgene. The highest expressing transgenic founder line produced human PLG corresponding to ~17% of the PLG level. Mice are generally highly resistant to subcutaneous infection by human GAS. However, introduction of human PLG expressed by the transgene markedly increased susceptibility to GAS. The increased susceptibility of *Tg+* mice to GAS was largely abrogated by deletion of the SK gene, demonstrating the major role of the PLG/SK interaction in GAS pathogenicity. We hypothesize that GAS hijack the host fibrinolytic system in order to circumvent local thrombosis for systemic spread. Consistent with this model, the marked difference in mortality between *Tg+* and *Tg-* mice was no longer observed when GAS were injected intravenously. Markedly increased mortality was also observed following GAS injection in C57BL/6J mice treated with the snake venom Ancrod, which proteolytically degrades plasma fibrinogen. In summary, activation of host plasminogen by SK leads to accelerated clearance of host fibrin and is a central mechanism for GAS invasion and spread. The remarkable species specificity of SK for host PLG probably resulted from host and pathogen coevolution. These observations highlight the potential role of infectious disease as a critical force in the evolution of the hemostatic system.

P-82**MULTIPLE MUTATIONS IN MOUSE *CHD7* PROVIDE MODELS FOR CHARGE SYNDROME**

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ENU mutagenesis Programs have yielded a number of mutants with dominant head-bobbing and circling behaviour due to truncations of the lateral semicircular canal of the inner ear, and the mutations map to proximal chromosome 4 (eg Kiernan *et al.* Mamm. Genome 13:142) . We have identified mutations in the *Chd7* gene in nine of these mutant alleles, including six nonsense and three splice site mutations. The human *CHD7* gene is involved in CHARGE syndrome (Vissers *et al.* Nat. Genet. 36:955), which also involves inner ear malformations plus a variety of other features with varying penetrance. We found widespread expression of *Chd7* in early development of the mouse in organs affected in CHARGE syndrome including eye, olfactory epithelium, inner ear, kidney and vascular system. Closer inspection of heterozygous mutant mice revealed a range of defects with reduced penetrance, such as cleft palate, choanal atresia, septal defects of the heart, oedema, haemorrhage, prenatal death, vulva defects and keratoconjunctivitis sicca. Many of these defects mimic the features of CHARGE syndrome.

P-83**MHC CLASS II DEFICIENCY REDUCES SUSCEPTIBILITY TO BLEOMYCIN-INDUCED PULMONARY FIBROSIS**

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Pulmonary fibrosis (PF) is a dose-limiting effect of bleomycin-based chemotherapy. Investigations using murine models determined that susceptibility to bleomycin is strain specific and that this strain specificity is partially genetically regulated. We examined the QTL *Blmpf1* in the MHC, which we previously found had a LOD=17.4 in both male and female mice in a genome wide scan. To narrow *Blmpf1* and identify candidate genes, we used three MHC congenic strains 4R, 5R and 2R on a susceptible C57BL/10SnJ background. %PF was significantly higher in 4R mice compared to 5R and 2R mice ($p < .001$, respectively). These data localized a susceptibility locus between 32.8 & 33.69Mb that includes candidate genes MHC Class I, II, and III genes but excludes the TNF gene cluster. MHC Class I and II knockout mice were then treated to further narrow the region. When knocked out, the Class II genes affect PF in the following three ways: there is an increase in the survival of mice, a decrease in the frequency of mice that have severe PF, and an increase in the frequency of mice with mild and moderate PF versus BL/6 controls. There were no differences in survival after bleomycin treatment and frequency of severity of PF between the control BL/6 mice and the Class I knockout mice, indicating that those genes are not susceptibility loci for BIPF. These data suggest that a MHC Class II gene(s) is involved in regulating PF, but the exact mechanism has yet to be determined.

P-84

MICROARRAY ANALYSIS OF GENE EXPRESSION DURING THE DEVELOPMENT OF DIABETIC NEPHROPATHY IN THE TYPE 2 DIABETES MOUSE MODEL LEPR^{DB/DB}

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In the UK diabetes affects more than 1.4 million people (3% total population) plus at least 1 million undiagnosed. Diabetes causes substantial morbidity and mortality mainly through cardiovascular disease, retinopathy, neuropathy, kidney disease and through limb amputation as a result of peripheral vascular disease.

Diabetic nephropathy is one of the most serious complications associated with this disease and is the number one cause of end-stage renal disease necessitating dialysis or transplantation.

We have used the type 2 diabetes mouse model *Lepr^{db/db}*, which develops diabetic kidney disease, in an expression profiling experiment using whole genome spotted oligo arrays. Expression profiles are being generated over a time-course of development of diabetic kidney disease and the analysis of this data set will be presented.

P-85

POLYA GENE TRAPPING AND NON-SENSE MEDIATED DECAY: MUTAGENESIS BY TARGETING TRAPPED GENES FOR DEGRADATION

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Gene trap mutagenesis of mouse embryonic stem (ES) cells efficiently generates random loss-of-function mutations, which can be identified by a sequence tag and can often report the endogenous expression of the mutated gene. An international gene trap consortium (IGTC), including our group (Centre for Modeling Human Disease, CMHD), was founded to generate a combined resource of gene trap insertions for the academic community. We have found that conventional gene trap vectors are limited in the range of genes that can be trapped, minimally due to a requirement of trapped gene expression in undifferentiated cells. We have developed a series of polyA trap vectors to determine if we can optimize trapping of genes not expressed in undifferentiated ES cells. In fact, our polyA trap vectors generate the most comprehensive set of unique gene trap events in the IGTC. Alas, we have also discovered that polyA trap insertions, often occur in the 3' end of genes. This occurs because most insertions in the 5' exons activate non-sense mediated decay (NMD), leading to an inability to select for 5' insertions. Thus, we generated a complement of gene trap vectors in which the polyA site of the reporter gene was deleted, leading to unstable trapped transcript, leading to hypomorphic mutations. We recently developed and tested novel vectors that overcome and utilize NMD to target the trapped gene for degradation. I will present our strategy, compare the CMHD gene trap library generation to the IGTC dataset, and discuss the pros and cons for our screen.

P-86**EFFICIENT GENE-DRIVEN GERM-LINE POINT MUTAGENESIS OF C57BL/6J MICE**

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Analysis of an allelic series of point mutations generated by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis is a valuable approach for discovering the full scope of the biological function of a gene. To facilitate a gene-driven approach for identifying ENU-induced mutations in any mouse gene, we produced the Cryopreserved Mutant Mouse Bank (CMMB), an archive of DNA, cDNA, tissues, and sperm from 4,000 G₁ male offspring of ENU-treated C57BL/6J males mated to untreated C57BL/6J females. High-throughput Temperature Gradient Capillary Electrophoresis (TGCE) was used to perform a 32-Mbp sequence-driven screen for mutations in 38 PCR amplicons from 11 genes in DNA and/or cDNA from the CMMB. DNA sequence analysis of heteroduplex-forming amplicons identified by TGCE revealed twenty-two mutations in 10 genes for an overall mutation frequency of 1 in 1.45 Mbp. All 22 mutations are single base pair substitutions, and nine of them (41%) result in nonconservative amino acid substitutions. Intracytoplasmic sperm injection (ICSI) of cryopreserved spermatozoa was used to recover mutant mice for nine of the mutations to date. The inbred C57BL/6J CMMB, together with TGCE mutation screening and ICSI for the recovery of mutant mice, represents a valuable gene-driven approach for the functional annotation of the mammalian genome and for the generation of mouse models of human genetic diseases.

P-87**A SENSITIZED ENU MUTAGENESIS SCREEN FOR DOMINANT GENETIC MODIFIERS OF THROMBOSIS IN THE FACTOR V LEIDEN MOUSE**

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Venous thrombosis affects ~300,000 individuals per year in the USA. A gain-of-function mutation in the factor V gene, Factor V Leiden, (FVL) is the most common known inherited risk factor for venous thrombosis. Penetrance is incomplete, with only ~10% of FVL individuals experiencing clinically significant thrombosis. We are performing a whole genome mouse mutagenesis screen to identify modifier gene candidates contributing to the penetrance of FVL in humans. Previously, we demonstrated synthetic lethality between FVL and genetic deficiency of a key coagulation component, tissue factor pathway inhibitor (TFPI). Complete TFPI deficiency in mice is embryonic lethal, whereas heterozygosity is compatible with normal survival. However, homozygosity for FVL (*Fv^{Q/Q}*) in the context of heterozygosity for TFPI (*Tfpi^{+/-}*) is uniformly lethal due to disseminated perinatal thrombosis. This synthetic lethal interaction was utilized as a phenotyping tool for a sensitized ENU mutagenesis screen. We aim to uncover novel dominant mutations that improve hemostatic balance leading to survival of *Fv^{Q/Q} Tfpi^{+/-}* mice. We have proven our approach by rescuing *Fv^{Q/Q} Tfpi^{+/-}* with tissue factor (*Tf^{+/-}*) heterozygosity. Male *Fv^{Q/Q}* mice were ENU mutagenized and bred to *Fv^{Q/+} Tfpi^{+/-}* double heterozygous females. Surviving G₁ offspring were analyzed to identify rescued mice with the *Fv^{Q/Q} Tfpi^{+/-}* genotype. Analysis of 3859 G₁ offspring thus far has identified 31 mice that survived to weaning. Of the 17 G₁ mutants progeny tested to date, 5 appear to be heritable. Mapping of the identified suppressor mutants is ongoing.

P-88

MOUSE MODELS OF HUMAN DISEASE: THE JACKSON LABORATORY REPOSITORY

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Mouse models of human disease are powerful tools to dissect the genetic pathways of disease pathogenesis. Efficient access to mouse models is essential as the use of mouse models continues to increase. Established as a resource for the scientific community, The Jackson Laboratory Repository imports, develops, preserves and distributes mouse strains vital to both basic and clinical research. 100 to 150 new strains are added annually to the approximately 2,800 strains and stocks of mice currently held in the Repository collection. Newly acquired or developed strains include models for Spinal Muscular Atrophy (SMA), Rett syndrome, Cystic Fibrosis, Diabetes, Huntington's disease, Alzheimer's disease, craniofacial disorders, kidney disease, vision and hearing disorders and spondyloepiphyseal dysplasia (SED). Strain information available from an on line resource (www.jax.org) includes brief phenotype descriptions, strain development and husbandry procedures, licensing requirements and a list of related references. Donating a strain to the Repository fulfills the requirements for sharing of mice in accordance with NIH's policy for the sharing of research reagents. Researchers can submit candidate strains using the on line form available at The Jackson Laboratory website at: <http://www.jax.org/grc/index.html>.

The Jackson Laboratory Repository is supported by the National Center for Research Resources (RR09781/RR11083), the National Institute on Aging, The Howard Hughes Medical Institute and private donations from several charitable foundations.

P-89

A NEW RECESSIVE X-LINKED MUTANT, BELLY HAIR LOSS (BHL) WITH STRAIN-SPECIFIC DIFFERENCES IN PHENOTYPE

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During an ENU mutagenesis screen, a new mutant arose that displayed progressive hair loss on the ventral side of the body, on the back of the ears, and around the eyes. As affected mice aged, they developed kyphosis and a kink at the end of the tail. The mutation has been genetically mapped to the region of the X Chromosome that contains Ectodysplasin-A (Eda). Mutations in Eda causes X-linked anhidrotic ectodermal dysplasia in humans. Although the hemizygous and homozygous mutants have similarities with alleles of *Ta*, *bhl*+/+ females exhibit the same phenotype as +/+ females. Comparison of mutants on the C57BL/6 background to F2 mice on the DBA/2J and CAST/EiJ backgrounds indicates significant differences in severity and age of onset. Details of mutant phenotypes in the three backgrounds, results of complementation testing with *Eda*^{Ta6J} and an update on efforts to detect the mutation responsible for the *bhl* phenotype will be described. The *bhl* mutant matches the phenotype of a hypothesized less severe allele of *Tabby*. The phenotypic differences found between *bhl* mice of different background strains could provide a basis for unravelling the mechanism that causes differences in disease severity.

P-90**NOVEL MODIFIERS OF APC^{MIN} ON CHROMOSOMES 18 AND 11**

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The Apc^{Min} mouse model of intestinal cancer develops discrete polyps, affording a quantitative measurement of tumor multiplicity. This property has previously been used to uncover two modifiers of the Apc^{Min} phenotype through quantitative trait locus analysis: Mom1 on distal chromosome 4 and Mom2 on medial chromosome 18. Here, we describe two new modifiers, Mom3 and Mom4, localized to chromosomes 18 and 11, respectively. Three inbred strains have been used: C57BL/6J.Apc^{Min} (~100 tumors), BTBR.Apc^{Min} (~500 tumors), and AKR/J.Apc^{Min} (~3 tumors). Reciprocal congenic substitutions of Mom4 between the B6 and AKR backgrounds demonstrate that homozygosity for the AKR allele provides a 1.5-fold resistance to tumorigenesis, fully recessive to the B6 allele. Strikingly, homozygosity for or compound heterozygosity between the AKR and BTBR alleles of Mom3 enhances intestinal neoplasia >2.5-fold in B6.Mom3 congenics. Conversely, one or two copies of the B6 allele of Mom3 reduces tumor multiplicity >2.5-fold in AKR.Mom3 and BTBR.Mom3 congenics. We hypothesize that Mom3 may act by directly modulating the rate of loss of heterozygosity (LOH) of the distal APC locus. The incomplete genome assembly of the pericentromeric region on chromosome 18 hinders the identification and characterization of Mom3, requiring novel methods of sequence assembly (see JM Amos-Landgraf, et al, abstract). It will be informative to determine whether direct regulators of LOH are also found on metacentrics (see LN Kwong, et al, abstract). The hypothesis that Mom3 may modulate the LOH of distal elements raises the possibility that the somatic LOH of canonical human tumor suppressors are similarly modulated.

P-91**EFFECTS OF COMBINED PERMEATING AND NON-PERMEATING CRYOPROTECTANTS ON SPERM QUALITY, DNA INTEGRITY AND IN VITRO FERTILITY IN DIFFERENT MOUSE STRAINS**

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Background: Efficient collection, freezing, archiving, and re-derivation of sperm are essential techniques to support large scale research programs using mouse models of human disease. The purpose of this study was to investigate the effect of novel cryoprotectants on post-thaw viability, DNA integrity, and fertility rate of frozen-thawed mouse sperm in order to optimise cryopreservation protocols.

Methods: Sperm was frozen using combinations of 3% skim milk + 0.2 or 0.3M nonpermeating raffinose with either permeating glucose, fructose, propylenglycol, ethylenglycol, glycerol, or sodium pyruvate in CD-1, C3FeB6F1/J, B6129SF1, C57BL/6NCrIBR, 129S/SvPaslco, and DBA/2NCrIBR mice. Sperm assessment parameters included progressive motility, plasma membrane integrity (SYBR-14+PI), *in vitro* fertility, and embryo development rates. DNA content analysis of sperm was measured by the *Sperm Chromatin Structure Assay* (SCSA).

Results: 0.3M raffinose with 0.1M fructose significantly improved post-thaw sperm quality for CD-1, C3B6F1, B6129SF1 mice ($P < 0.05-0.01$), whereas 0.2M raffinose with 0.1M glycerol or 0.1M fructose enhanced sperm quality for C57BL/6 and 129S mice, compared to 0.3M raffinose alone. DNA fragmentation during cryopreservation was significantly increased in all strains evaluated compared to fresh control sperm in a strain-dependent manner. Supplementation of permeating glycerol or fructose to the CPA solution showed a significant protective effect to DNA integrity when cryopreserving sperm from C57BL/6 and 129S mice. Damage of sperm DNA decreased significantly the rate of embryo development to blastocyte in C57BL/6 mice.

Conclusions: Type of monosaccharide sugar or polyols, CPA molarity, and combination of permeating and nonpermeating cryoprotectant are important factors for efficient cryopreservation of mouse sperm.

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A NEW MOUSE MUTANT FOR THE LDL RECEPTOR

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We have identified a new mouse mutant for the LDL receptor from an ENU mutagenesis screen using high throughput phenotyping. From a third-generation screen using background strain C57BL/6J, four likewise affected "G3" animals from 13 progeny of a common G1 founder were originally identified on the basis of an elevated total plasma cholesterol in response to having consumed a high fat and high cholesterol diet for five weeks. Affected animals had an average 4.5-fold increase in cholesterol from baseline (consuming standard chow) whereas strain C57BL/6J typically exhibits an average 2.5-fold increase in response to the same diet. Heritability of the phenotype was confirmed and genetic mapping suggested the LDL receptor as a candidate gene. Sequencing revealed that the point mutation is a G to A transition causing replacement of a cysteine by tyrosine in exon 14. This mutation, while previously described in humans, is novel in the mouse. A PCR-based assay was developed to identify homozygous mutants and thereby establish a colony. On further analysis of homozygous mutants, we have observed that this mutation causes severe atherosclerotic plaques containing collagen in the aorta, atheromas in coronary and pulmonary arteries, elevated HDL as well as total plasma cholesterol, increased blood urea nitrogen, and retinal degeneration and retinal detachment. This mutant is a robust new tool for use in understanding the process of atherosclerosis.

P-93

RENAL AND ADRENAL EFFECTS OF ACTH EXCESS

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ACTH-dependent Cushing's Syndrome is characterised by central obesity, insulin resistance and hypertension. Arguably hypertension is caused by excess adrenal steroids acting via mineralocorticoid (MR) or glucocorticoid (GR) receptors or a combination of both. Mice are a good species to model Cushing's Syndrome because, unlike rats, they are not unduly sensitive to the catabolic effects of glucocorticoids. Accordingly we have tested the renal and adrenal effects of a two week sc infusion of ACTH (Synacthen 2.8 microgram/d) in groups of male C57BL/6 mice. Blood pressure was increased by 20mmHg, food intake by 20% and fluid intake by 350% and adrenal weight was more than twofold greater ($P < 0.02$). Body weight gain was unaffected. (An equivalent dose of ACTH in rats also increased blood pressure but caused marked weight loss). Signs of mineralocorticoid excess included hypokalaemia and hypernatremia ($P < 0.001$) but volume expansion was not apparent (haematocrit was increased not decreased; $P < 0.02$). Since we have shown previously that aldosterone-induced hypertension in this strain of mouse is dependent on large doses of steroid, a high sodium diet and reduced renal mass, it seems unlikely that ACTH increases blood pressure solely through MR. Decreased thymus weight (55% less, $P < 0.01$) indicates that glucocorticoid activity is greater too. Renal function studies showed elements of both MR and GR effects. Fractional excretion of sodium was 50% less with ACTH (MR) but so too was potassium excretion ($P < 0.05$). GFR, a glucocorticoid-dependent variable, was 70% higher ($P < 0.001$). Amiloride-sensitive sodium excretion tended to be higher (MR) but the effect was not statistically significant. We conclude that adrenal steroids act in tandem via MR and GR to increase blood pressure in ACTH excess.

P-94**MDM MUSCULAR DYSTROPHY: INTERACTIONS WITH CALPAIN 3 AND A NOVEL FUNCTIONAL ROLE FOR TITIN'S N2A DOMAIN**

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Human tibial muscular dystrophy (TMD) and limb-girdle muscular dystrophy 2J (LGMD2J) are caused by mutations in the giant sarcomeric protein titin (TTN) adjacent to a binding site for the muscle-specific protease calpain 3 (CAPN3). Muscular dystrophy with myositis (*mdm*) is a recessive mouse mutation with severe and progressive muscular degeneration caused by a deletion in the N2A domain of titin (TTN-N2A^{Δ83}), disrupting a putative binding site for CAPN3. To determine whether the muscular dystrophy in mutant *mdm* mice is caused by misregulation of CAPN3 activity, genetic crosses with CAPN3 overexpressing transgenic (C3Tg) and CAPN3 knockout (C3KO) mice were generated. Here we report that overexpression of CAPN3 exacerbates the *mdm* disease, leading to a shorter lifespan and more severe muscular dystrophy. However, in a direct genetic test of CAPN3's role as a mediator of *mdm* pathology, C3KO;*mdm* double mutant mice showed no change in the progression or severity of disease indicating that aberrant CAPN3 activity is not a primary mechanism in this disease. To determine whether we could detect a functional deficit in titin in a non-disease state, we examined the treadmill locomotion of heterozygous *+/mdm* mice and detected a significant increase in stride time with a concomitant increase in stance time. Interestingly, these altered gait parameters were completely corrected by CAPN3 overexpression in transgenic C3Tg;*+/mdm* mice, supporting a CAPN3-dependent role for the N2A domain of TTN in the dynamics of muscle contraction.

P-95**RECESSIVE BONE MARROW DEFECTS AND LIVER DYSPLASIA IN ENU INDUCED MUTANT CCH001 MICE**

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Within the Munich ENU mouse mutagenesis project, the recessive mutant mouse line CCH001 was established showing major hematological and clinical-chemical abnormalities. Homozygous mutant animals displayed severe anemia and thrombopenia with marked anisocytosis at the age of twelve weeks combined with extremely elevated liver enzyme activities. Pathological examination revealed a depleted bone marrow in the mutant mice after six weeks post partum (p.p.). Simultaneously, excessive extramedullary hematopoiesis was observed in liver and spleen. After eight weeks p.p., the liver developed nodular hyperplasia progressing to adenoma or hepatocarcinoma tumors in older mice. Affected mice died after 4-6 months p.p. due to severe anemia leading to cardiomegaly and cardiac infarction. Most of the heterozygous mutant animals showed no pathologic changes except for increased levels of alkaline phosphatase activity in plasma. Genome-wide polymorphic microsatellite analysis in backcross animals resulted in the mapping of the causative mutation to mouse chromosome 6 in the range of 40-50 Mb. Comparative genome analysis was carried out for the search of candidate genes and detected a genetic defect in humans associated with impaired hematopoiesis. The myelodysplasia and leukemia syndrome including monosomy 7 is caused by chromosomal aberrations in the syntenic region of the mapped locus. Further examinations of line CCH001 will include the identification of the mutated gene and the detailed pathogenesis of the mutant phenotype giving rise to increased knowledge about the regulation of hematopoiesis.

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ROLE OF AP-2g IN ERBB-2-INDUCED TUMORIGENESIS

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A causative role of the membrane-bound tyrosine kinase ErbB-2 in breast tumorigenesis has been well established. MMTV/neu transgenic mice which overexpress ErbB-2 consistently develop mammary carcinomas with a high incidence. In human breast cancer, ErbB-2 is overexpressed in 25 % - 30% of all cases and is representing a clinical marker of a poor prognosis. Besides to gene amplification, ErbB-2 overexpression has been attributed to transcription factors of the AP-2 family which were shown to control the erbB-2 gene promoter in cell culture studies. Particularly AP-2g and g are often coexpressed in ErbB-2-positive breast carcinomas. However, LTRg transgenic mice which overexpress AP-2g in their mammary epithelium display only a very weak upregulation of the erbB-2 gene and do not develop mammary carcinoma. These findings therefore raise the possibility of functional cooperation between both genes in breast cancer. To experimentally address the impact of AP-2g on ErbB-2-induced breast carcinogenesis we crossed MMTV/neu transgenic mice with LTRg transgenic mice and monitored tumor development in bitransgenic female progeny. AP-2g overexpression negatively influenced tumor incidence, as reflected by a reduced tumor number and prolonged tumor latency. Histological analysis revealed three major types of tumours corresponding to different stages of tumor progression. Interestingly, an increased proportion of advanced stage carcinomas was observed in bitransgenic mice. Moreover, the AP-2g transgene differentially affected proliferation rates between the different progression stages: proliferation was enhanced at early stages but reduced in advanced stages in comparison to control tumours. Therefore, AP-2g while reducing the incidence of mammary tumours is promoting tumor progression. In the tumor samples tested we have observed a constitutive and strong expression of endogenous AP-2g raising the possibility that it is required for tumor development or crucial for progression. Our current attempts aim at resolving this issue using conditional AP-2g mutant mice crossed with MMTVcre and MMTV/neu transgenic mice

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