

Tuesday November, 14
10.30am – 12.30pm
Poster Session 3
Modeling Disease by Genome Manipulation/Mutagenesis
Posters P67 – P89

P67 CHARACTERIZATION AND HIGH RESOLUTION MAPPING OF LUCA, A NEW MOUSE HAIR LOSS MUTATION

CJ Perez¹, L Mecklenburg², E Mirabzadeh³, I Aubin³, BM Iritani⁴, T Habib⁴, H Park⁴, J Blando¹, O Contreras¹, CJ Conti¹, J DiGiovanni¹, J-L Guénet³, FJ Benavides¹

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S6/ IN VIVO AND MOLECULAR CHARACTERIZATION OF BONE RELATED PHENOTYPES

P68 F Thiele, T Lisse, GKH Przemec, H Fuchs, M Hrabé de Angelis

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S4/ CHARACTERIZATION OF A NOVEL GENE TRAP DERIVED CHD7 ALLELE DEMONSTRATES EMBRYONIC
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EA Hurd, HK Poucher, PL Capers, DM Martin

University of Michigan, Ann Arbor, MI, United States

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P70 FORMATION

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P71 EPIGENETIC REGULATION OF INSULIN RESPONSIVE GLUCOSE TRANSPORTER GLUT4 IN ADULT IUGR SKELETAL MUSCLE

N Raychaudhuri, S Raychaudhuri, M Thamocharan, S Devaskar

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RJ Westrick¹, SL Manning², ME Winn¹, SL Dobies¹, GM Stotz¹, DR Siemieniak², E Sanford², D Ginsburg²

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P73 AN IMPROVED CO-CULTURE TECHNIQUE FOR GENERATING TRANSGENIC MICE BY LENTIVIRAL INFECTION PRODUCES BOTH WHOLE-BODY AND PROSTATE-SPECIFIC LUCIFERASE EXPRESSION IMAGED BY A NON-INVASIVE IN VIVO IMAGING SYSTEM

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P74 CHARACTERIZATION AND MAPPING OF ALI34: A NEW ENU-DERIVED MURINE MODEL FOR OSTEOARTHRITIS

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T Schmitt-John¹, A Mussmann¹, D Lasrich², P Heimann², M Ulbrich², C Drepper¹

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L Li¹, EM Rinchik², SE Mentzer¹, D Carpenter¹, RD Nicholls³, DK Johnson¹, Y You¹
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P89 A MOUSE MODEL OF ER-TO-GOLGI PROTEIN TRANSPORT DEFICIENCY

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CHARACTERIZATION AND HIGH RESOLUTION MAPPING OF LUCA, A NEW MOUSE HAIR LOSS MUTATION

CJ Perez¹, L Mecklenburg², E Mirabzadeh³, I Aubin³, BM Iritani⁴, T Habib⁴, H Park⁴, J Blando¹, O Contreras¹, CJ Conti¹, J DiGiovanni¹, J-L Guénet³, FJ Benavides¹

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In this study we present *luca* (*luc*), a new mouse mutation associated with hair loss. We determined that *luc* is an autosomal recessive mutation and we mapped the locus to chromosome 7. Affected *luc/luc* mice develop generalized hypotrichosis and multifocal patchy alopecia, starting at postnatal day 7. Hair shaft dysplasia is characterized by twisting and multifocal pigment clumping, suggesting defects in the complex differentiation program of trichocytes. At postnatal day 22, the number of anagen II hair follicles appears to be reduced in homozygous mice, whereas in wild type and heterozygous mice all hair follicles undergo their first true anagen phase. At postnatal day 30, all hair follicles in *luc/luc* mice have very prominent sebaceous glands, suggesting that anagen induction occurs in tylotrich hair follicles only, whereas non-tylotrich hair follicles likely remain in telogen or undergo atrophy. Multifocal alopecia and erythema in homozygous mice is also associated with inflammatory cell infiltration. No further relevant organ abnormalities were detected on histopathology of several organ systems. In a preliminary immunological screen, no differences were identified between *luc/luc* mice and littermate controls. We report the genotyping of 300 F₂ mice (600 meioses) that allowed us to narrow down the segment of chromosome 7 containing *luc* to a 1 Mb interval between markers *D7Mit272* and *D7Mit93* (45-46 Mb), a region of homology with human chromosome 11p15.1. Positional candidate genes for the *luc* mutation include *Zdhhc13*, *Csrp3*, *Dbx1*, and RIKEN cDNA 5330421F07. The *luc* mutation represents a potential new model for alopecia, hair follicle cycling and trichocyte differentiation.

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EPIGENETIC REGULATION OF INSULIN RESPONSIVE GLUCOSE TRANSPORTER GLUT4 IN ADULT IUGR SKELETAL MUSCLE

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Background: Adult intra-uterine growth restricted (IUGR) offspring is predisposed to type 2 diabetes. The molecular basis is decreased skeletal muscle (SM) glucose transporter (GLUT4). MyoD/MEF2 bind and activate GLUT4 transcription. Epigenetic control consists of heterochromatin formation that negatively affects MyoD/MEF2 binding and decrease in GLUT4 transcription.

Hypothesis: Decreased SM GLUT4 expression in the IUGR offspring is regulated by epigenetic mechanisms.

Methods: CpG islands regulating GLUT4 transcription were detected by luciferase activity in transient transfection assays using wild type or mutated GLUT4-luciferase constructs in L6 or C2C12 SM cells (n=6 each). DNA methylation of the GLUT4 promoter was assessed by bisulphite sequencing. Employing chromatin immunoprecipitation (ChIP) assays involving CpGs, MyoD and MEF2 sites, the histone code was deciphered in SM chromatin of 450d rats born to and reared by control (CON; n=4) or 50% nutrient restricted mothers (IUGR; n=4). The interaction of chromatin modifying enzymes was also evaluated. The presence of a repressor complex was confirmed by ChIP-ReIP.

Results: CpG islands near MyoD/MEF2 site inhibited *Glut4* transcription (p<0.005). SM MyoD and MEF2 binding to GLUT4 was decreased in IUGR (p<0.05). H3 was deacetylated on K14 (p<0.05) and dimethylated on K9 in IUGR. HDAC1 binding to GLUT4-MyoD complex increased at MyoDII site and HDAC4 binding increased at the MEF2 site in IUGR. While SUV39H1-GLUT4 interaction increased in IUGR, HP1 binding remained the same.

Conclusions: In adult female IUGR offspring, H3-K14 deacetylation, and H3-K9 dimethylation precludes efficient MyoD/MEF2-GLUT4 binding and ultimately decreases SM GLUT4 expression

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AN IMPROVED CO-CULTURE TECHNIQUE FOR GENERATING TRANSGENIC MICE BY LENTIVIRAL INFECTION PRODUCES BOTH WHOLE-BODY AND PROSTATE-SPECIFIC LUCIFERASE EXPRESSION IMAGED BY A NON-INVASIVE *IN VIVO* IMAGING SYSTEM

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The use of lentiviral vectors for producing transgenic mice represent a more rapid and less labour intensive procedure, compared with pronuclear injection of transgenic vectors. Here we describe the generation of transgenic mice by infection of embryos with a lentiviral vector encoding firefly luciferase, allowing non-invasive *in vivo* imaging using the Xenogen imaging system. Embryos denuded of their zona pellucida were individually cultured in micro-depressions on a culture plate within microdrops of media, allowing simultaneous culture of a large number of embryos. Transgenic efficiency correlated predictably with viral titer. Almost 100% efficiency was achieved using a viral titer of 10⁵ international units (I.U.) per microliter (determined by p24 ELISA) in co-culture with the embryos. Transgenic mice expressing luciferase either ubiquitously or in a tissue specific manner were produced. Furthermore, F1 embryos expressing luciferase under the ubiquitin C promoter were visualized as early as embryonic day 8 in female mice impregnated by transgenic stud males. Transgenic mice exhibiting tissue specific expression of luciferase under the prostate-specific promoter ARR2PB were generated, which allows real-time *in vivo* imaging to monitor the growth and size of the prostate. This technique can be used for evaluating tumor burden in mouse prostate cancer models, and allows non-invasive *in vivo* monitoring of metastasis.

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CHARACTERIZATION AND MAPPING OF ALI34: A NEW ENU-DERIVED MURINE MODEL FOR OSTEOARTHRITIS

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We have recently utilized the Munich N-ethyl-N-nitrosourea (ENU) mutagenesis program in an attempt to systematically identify new genes and alleles that control events during skeletal development and homeostasis in mouse (1,2). From this platform, an autosomal dominant mutant line termed *ALI34* (abnormal limb 34) was isolated depicting osteophytes and chondrocytic developmental abnormalities in the patello/tibio-femoral joints and both articular and growth plate cartilage anlage, respectively. We have investigated the *ALI34* manifestation during adult stages, whereby *ALI34*+/+ animals exhibit shortened and increased mineralization in bi- or uni-lateral hind limbs, and an increased synovial mass. Furthermore, progressive loss of articular chondrocytes (chondromalacia) is apparent, and disorganized clusters of large hypertrophic chondrocytes and horizontal bone struts are present within and at the base of the growth plate, respectively, preventing longitudinal growth; meanwhile appositional bone growth remains unaltered. High throughput SNP genotyping was applied for linkage analysis by outcrossing to C57BL/6 and then backcrossing to originating C3HeB/FeJ as was complicated by severe modifier effects (3). Significant *ALI34* linkage was observed on chromosome 5, in which further haplotype analysis is currently being conducted. Osteoarthritis (OA) is the most common non-inflammatory disease of synovial joints in humans, and *ALI34* phenotypically displays the hallmarks of OA. The identification of the *ALI34* locus may represent a novel skeletal gene which will enlighten our understanding of skeletal regulation and disease outcome.

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LARGE-SCALE MAPPING STRATEGY IN A LARGE-SCALE MUTAGENESIS SCREEN

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The Munich ENU-Mouse Mutagenesis Screen was established for large-scale and genome-wide production and analysis of mouse mutants as model systems for inherited human diseases with new main focus on bone and cartilage diseases. For this purpose bone densitometry and a blood screen for bone parameters and bone turnover markers have been implemented in the F1 screen. Additionally a sensitized screen with the *Dll1* heterozygous knockout mouse has been set up in order to identify modifiers of the Delta-Notch pathway. First mutants have already been isolated and will be presented. High-speed backcross mapping by IVF and embryo transfer of established mouse lines from the sperm archive has been implemented. To date more than 730 recessive and dominant mutant mouse lines have been established in the genome-wide screen, 132 mutant lines have been mapped and 54 have been cloned and published so far. Considering the high number of mutant mouse lines a systematic mapping of all available mutant mouse lines has become indispensable.

A genotyping platform has been established using MALDI-TOF mass spectrometry for high-throughput mapping. 384 individuals representing up to eight different mouse lines can be analyzed within one week by employing a panel of 150 SNP markers evenly distributed over the entire genome. 35 mutant lines have finished SNP analysis since November 2004. As an example of a new model for scoliosis the recessive mutant mouse line KTA041 with first mapping results will be presented.

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VPS54 ALLELES GIVE NEW INSIGHT IN THE PATHOMECHANISM OF AMYOTROPHIC LATERAL SCLEROSIS

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The wobbler mouse is an intensively investigated animal-model for human motor neuron diseases such as amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). The positional cloning of the *wobbler* mutation identified *Vps54* to be critical for motor neuron degeneration (1). The wobbler gene, *Vps54*, encodes a highly conserved vesicle traffic factor that is a component of the "GARP" (Golgi-associated retrograde Protein) complex. This protein complex is involved in the retrograde vesicle transport from late endosomes to the trans Golgi network. In wobbler mice a single amino acid exchange in the c-terminus of *Vps54* leads, besides the signs of neurodegeneration, to enlarged endosomal structures in motor neurons and to an impairment of the retrograde vesicle transport. In a subset of sporadic early-onset ALS patients we found similar enlarged endosomes, indicating that the retrograde vesicle traffic is critical, at least for a subset of ALS cases.

In many different neurodegenerative disorders protein aggregations appear to be involved in the pathomechanism. For ALS neurofilament aggregations have been reported. Here we demonstrate aggregations of the intermediate filaments neurofilament and vimentin in wobbler motoneurons that are involved in or contribute to the pathomechanism of the motor neuron degeneration.

Since *wobbler* appears to be a hypomorphic *Vps54* allele we generated a null allele, which turned out to be embryonic lethal around day 11 of the embryonic development. Heterozygous *Vps54* +/- mice appeared to be viable and fertile, but in 20% of the cases heterozygotes displayed a motor neuron disease very similar to wobbler, except that surprisingly hindlegs are first affected rather than forelegs, as is the case for wobbler mice.

(1) Schmitt-John et al., (2005) Nature Genetics, 37, 1213-1215.

P77**HOMOZYGOSITY FOR TWO N-ETHYL-N-NITROSOUREA (ENU)-INDUCED AND A GENE-TRAP MUTATIONS OF CYTOPLASMIC FMRP-INTERACTING PROTEIN 1 (CYFIP1) DISRUPTS MOUSE EMBRYO DEVELOPMENT**

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The functional unit *I71Rl* has been defined as a peri-implantation lethal locus by complementation analysis of deficiencies in the *p*-region of mouse Chromosome 7. Previous characterization of the genomic region indicated that the locus encompasses the Cytoplasmic FMRP (Fragile-X Mental Retardation Protein)-interacting protein 1 (*Cytip1*), also known as *Shyc* or *Sra-1*, and three other genes. Homozygous *Cytip1*^{GT/GT} embryos, generated from a *Cytip1* gene trap (*GT*) ES cell line with a trap vector inserted between exon 12 and exon 13, implanted with apparently normal development until 7.5-8.5 days post coitum (dpc), after which development was arrested without further anterior-posterior elongation. The mutant embryos remained on the lordotic position and were disintegrated after 10.5dpc. Two N-ethyl-N-nitrosourea (ENU)-induced recessive lethal mutations, *Cytip1*^{enu1R} and *Cytip1*^{enu2R} (stock785DSJ and 828DSJ respectively) were mapped within the *I71Rl* critical interval. Inter- and intra-crosses of the two ENU-induced mutations and heterozygous *Cytip1*^{GT/+} mice revealed that the ENU-induced and *Cytip1*^{GT} alleles cannot complement each other and the homozygous or compound heterozygous mutants displayed similar embryonic abnormalities as the homozygous *Cytip1*^{GT/GT} embryos. The results unambiguously indicated that these three mutations are allelic. Single nucleotide substitutions within *Cytip1* gene were identified in genomic DNA of *Cytip1*^{enu1R} and *Cytip1*^{enu2R} alleles, which led to in-frame exon skipping of exon 5 and exon 27 respectively. The lethality of homozygous *Cytip1*^{GT/GT}, *Cytip1*^{enu1R/enu1R} and *Cytip1*^{enu2R/enu2R} embryos indicates that a structurally intact protein is indispensable during mouse embryonic development. The function of *Cytip1* in mouse development is being analyzed by molecular and cellular approaches.

P78**FUNCTIONAL CHARACTERIZATION OF SCNM1, A DISEASE MODIFIER, BY GENERATION OF A CONDITIONAL FLOXED ALLELE**

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SCNM1 (sodium channel modifier 1) is a zinc finger protein and putative RNA splicing factor that was identified as a modifier of the sodium channel allele *Scn8a-medJ* (Buchner et al, Science 2003). The SCNM1 allele R187X is carried by strain C57BL/6J and closely related strains, and impairs the efficiency of splicing of a weak splice donor site. To further characterize the function of SCNM1, we targeted the gene with flox sites flanking exons 3 and exon 5 to delete the zinc finger domain of the protein. Floxed mice were crossed with mice carrying the EIIA CRE transgene, for ubiquitous deletion of exons 3 to 5. Homozygotes for the deleted allele are viable and fertile, and produce a stable deleted protein. RNA from deleted and wildtype mice are being compared by microarray analysis, to detect additional substrates of SCNM1. Altered transcripts have been detected in liver of deleted mice. We are using primary fibroblasts from the deleted mice for a functional assay of SCNM1 activity, monitoring splicing of a transfected sodium channel minigene. We have also carried out yeast-two hybrid analysis to identify proteins interacting with SCNM1. Two proteins interacting proteins have been identified, one of which is the mammalian ortholog of a yeast spliceosome protein. Our preliminary results support a role for SCNM1 in mRNA splicing, a likely mechanism for its trans-action as a disease modifier gene.

P79**A RANDOM INSERTION OF A ROSA26-EGFP TRANSGENE LEADS TO MALE INFERTILITY IN MICE**

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In this study, we have characterized a mouse model of male infertility which occurred in a mouse line containing a ubiquitously expressed EGFP transgene. Males homozygous for the mutation were unable to produce pups by either natural mating or *in vitro* fertilization; however, homozygous females had normal fertility. Attempts to isolate spermatozoa from the epididymis using swim out methods were unsuccessful. Spermatozoa recovered by manual methods from the epididymis lacked motility and exhibited ~80% abnormal head and midpiece defects. Histological analysis of the testes and epididymis revealed disorganization of the seminiferous tubules, large vacuoles within seminiferous tubules and the epididymis as well as some sloughing of epithelium within the seminiferous tubules and occasional giant cells. Immunohistochemistry performed for GATA4, which stains sertoli cell nuclei, demonstrated that vacuoles are located within sertoli cells of the seminiferous tubules. Utilizing a chromosome walking technique, we have mapped the transgene insertion site to Chromosome 3 within an intronic region of a novel predicted gene (ENSMUSG00000074463). Based on nucleotide sequence data, at least two copies of the transgene, consisting of the enhanced green fluorescent protein (EGFP) under the control of the ubiquitously expressed Rosa26 promoter, have been incorporated in inverted repeat orientation associated with higher recombination rates. Interestingly, we have identified a male with recombination in which EGFP expression is now restricted to the testes. Using EGFP as a visible marker, it will be possible to further characterize this unique model which will provide insight into male fertility as well as spermatogenesis.

P80**COMPARISON OF ENU-INDUCED MUTATION-DISCOVERY SYSTEMS FOR SEQUENCE-BASED MUTAGENESIS IN THE MOUSE**

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In the RIKEN ENU mouse mutagenesis project, we have produced the frozen sperm archive from over 8000 G1 male offspring. All the corresponding G1 genomic DNAs have also been archived and thus the sequence-based mutagenesis for particular genes of the interest has become available. This system can be used as a reverse genetics tool to analyze the gene function through various mutant mice. To detect the mutation in target genes from the archive, we have adopted the TGCE method that can efficiently distinguish heteroduplex from homoduplex DNA fragments. We are now able to detect approximately 100 point mutations per year. To enhance the mutation detection efficiency and to accelerate the functional gene analysis in the entire mouse genome, we have been comparing several mutation detection methods. For this purpose, we firstly produced positive control samples, each of which carries a single base substitution in a mouse genomic DNA sequence. We have constructed about 500 such positive controls for 24 different sequences from the mouse genome. Our report encompasses the comparison among TGCE, Tilling and Hi-res melting methods for the detection of point mutations. The sensitivity and efficiency for the mutation detection as well as the throughput and cost performance of each method were compared using the positive controls. The comparison was also made by combining the pooling genomic DNA samples to raise the whole throughput of the screening.

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THE SANGER INSTITUTE MOUSE GENETICS PROGRAMME: HIGH THROUGHPUT GENERATION AND CHARACTERISATION OF KNOCKOUT MICE

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The Wellcome Trust Sanger Institute is embarking on an extensive gene/phenotype-driven mutagenesis programme. We aim to generate, characterise and archive up to 2500 new lines of knockout mice over the next 5 years. In addition to studying the role of each gene in normal development and function, the breadth and depth of our phenotyping platform will ensure that phenotype information on a wide spectrum of disease conditions will be obtained for each individual mouse line without the need for any prior assumptions about function. Disease indications focused on include cancer, obesity, diabetes, infertility, changes to sensory acuity, kidney and cardiovascular disease, developmental and musculo-skeletal problems. Expression data will also be collected for each target gene. Following validation and stringent quality control all data will be deposited on an open access web based browser. Furthermore, all biological resources generated by the programme (mice, ES cells and targeting vectors) will be openly available to the scientific community. Contributions to the programme in the form of target gene requests or phenotypic screen proposals are encouraged.

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HIGH-EFFICIENCY GENE TARGETING WITH C57BL/6J EMBRYONIC STEM CELLS ESTABLISHED IN A DEFINED SERUM-FREE MEDIA

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C57BL/6J is a well-characterized mouse strain that is used extensively for immunological and neurological research. The establishment of C57BL/6 ES cell lines has facilitated the study of gene-altered mice in a pure genetic background; however, relatively few such lines exist. Using a defined media supplement, Knockout Serum Replacement™ (KSR) with Knockout™ DMEM (KSR-KDMEM), we find that we can readily establish ES cell lines from blastocysts of C57BL/6J mice. Six lines were established, all of which were karyotypically normal and could be maintained in the undifferentiated state on mouse embryonic fibroblast (MEF) feeders. One line has been tested with 15 different DNA targeting constructs and we have achieved an average of 18% homologous recombination. For this cell line, efficiencies of cell cloning and chimera generation were greater when maintained in KSR-KDMEM. All constructs tested have been transmitted through the germline with the one exception of an X-linked gene that lead to a premature lethal phenotype in the chimeras. Our work suggests that use of defined serum-free media may facilitate the generation of ES cells from inbred mouse strains and increase the efficiency of gene targeting.

P83**SUCCESSFUL AND CONSISTENT CRYOPRESERVATION AND RECOVERY OF FROZEN SPERM FROM INBRED MOUSE STRAINS**A Chen, L Garrett-Beal

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Embryo freezing has been the conventional method of preserving mutant strains because of its reliability. This approach, however, requires many mice for adequate archiving, costly equipment, and is time & labor intensive. When sperm cryopreservation was established, it offered a more economical method that required fewer mice, as well as a rapid freezing procedure. However, the efficiency of recovery of frozen sperm from mutant mouse strains by *in-vitro* fertilization has been low and inconsistent for some inbred strains (e.g. C57Bl6/J, 129S6/SvEv). In this study, we compared the *in-vitro* fertilization rates from frozen sperm of several strains that were used to produce transgenic mice in our laboratory: FVB/NTac, C57Bl6/J, and 129S6/SvEv. Initial average IVF rates ranged from 90% for FVB/NTac, 10-20% for 129S6/SvEv, and 5-10% for C57Bl6/J.

To improve the IVF rates for C57Bl6/J and 129S6/SvEv strains, we compared different methods of treatment. We find that by using IVF media (HTF) with an optimal osmolarity of 294-296±2mOsm, fertility rates increased for all the strains tested. In addition, treating the zona-pellucida of oocytes with Tyrode's Acid solution prior to IVF increased the IVF rates for C57Bl6/J to 40-90%, but did not further increase rates for 129S6/SvEv. The ability to efficiently reconstitute mutant mice from frozen sperm with these inbred strains will greatly enhance a cryopreservation program.

P84**A NONSURGICAL TRANSCERVICAL METHOD FOR MOUSE EMBRYO TRANSFER**MA Green, BT Spear

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Mice have proven to be invaluable in biomedical research. To a large extent, this is due to our ability to manipulate the mouse germline through transgenic and gene knock-out technologies. Despite the value of gene-modified mice, it is clear that research with mice is becoming more complicated. Per diem charges continually increase, and paperwork required for IACUC approval has become more time-consuming. In regards to genetically modification of mice, particular challenges are associated with the surgical transfer of embryos. In addition to IACUC concerns associated with surgery, substantial training is needed to become proficient in uterine and oviduct surgery, specialized, sterile surgical equipment is required, and potential problems associated with anesthesia and post-operative recovery exist, including possible infection and the time spent to monitor animals to insure that recovery proceeds without complications.

To overcome these problems, we have developed a non-surgical, transcervical technique for embryo transfer. A catheter has been developed for transcervical delivery into pseudopregnant female mice. The entire procedure takes several seconds and does not require anesthesia. For transgenic production, injected embryos are allowed to develop to blastocysts prior to non-surgical transfer. The number of pups born, and those that are transgenic, are comparable to the numbers obtained using standard surgical transfer. We have also transferred chimeric embryo aggregates using the transcervical transfer, suggesting that this technology can also be used for ES cell chimeras. This technique could serve as an improvement over the current surgical methods of embryo transfer that are currently being used.

P85**PRIMARY CILIUM SIGNALING IS ESSENTIAL FOR HAIR FOLLICLE MORPHOGENESIS**J M Lehman¹, E Laag², J P Sundberg³, E J Michaud², B K Yoder¹¹Department of Cell Biology, University of Alabama at Birmingham, Birmingham, United States, ²Mammalian Genetics and Genomics Group, Oak Ridge National Laboratory, Oak Ridge, United States, ³The Jackson Laboratory, Bar Harbor, United States

The primary cilium is a microtubule-based sensory organelle present on most mammalian cells. Genetic mutations that disrupt the function of primary cilia result in a broad spectrum of disorders, including polycystic kidney disease and skeletal abnormalities. Recent studies showed that the primary cilium is essential for mediating sonic hedgehog (Shh) signaling during neural tube patterning, left-right axis specification, and limb development. Here we demonstrate that primary cilia are also essential for Shh signaling during hair follicle morphogenesis. We used a Cre-loxP strategy to selectively knock out the primary cilia genes *Ift88* and *Kif3a* in dermal fibroblasts and dermal papillae of mice, and compared their hair follicle phenotypes and cilia distribution to wild-type animals. In wild-type mice, cilia were present on most cells of the hair follicle in an apical orientation in the developing hair bud and germ. Primary cilia were particularly prominent on the dermal papillae and matrix cells of the adult hair follicle. In *Prrx1-cre;Ift88^{flox}* mice, where the *Ift88* gene was deleted in dermal papillae and dermal fibroblasts of the ventrum and limbs, most hair follicles were arrested at the hair germ stage, consistent with a block in Shh signaling. Primary cilia were also absent on the dermal papillae and dermal fibroblasts. The same results were obtained with *Prrx1-cre;Kif3a^{flox}* mice. These data suggest an essential role for dermal primary cilia in reception of the epidermal Shh signal during hair follicle morphogenesis.

P86**CHARACTERIZATION OF BEHAVIORAL MUTANTS IDENTIFIED IN THE GNF ENU NEUROSCREEN**JS Bailey, L Grabowski, BM Steffy, SW Barnes, T Wiltshire, LM Tarantino
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We have carried out a forward-genetic behavioral screen to identify phenotypic outliers in the progeny of ENU-mutagenized C57BL/6J mice. High-throughput phenotyping assays were conducted to identify mutants with sensorineural defects as well as those with abnormal responses in assays measuring complex behaviors. Using the open-field assay, we identified mutants with heritable alterations in activity level and anxiety-related behaviors. Several of these lines also show abnormal responses to dopamine-modulating drugs. The mutant line *Highper* is hyperactive and exhibits an exaggerated locomotor response to cocaine and methylphenidate. Interestingly, this line also shows abnormally prolonged elevation of corticosterone levels following restraint stress. We are currently investigating whether this apparent hypothalamic-pituitary-adrenal axis dysfunction and the hyperactivity and drug-response phenotypes in *Highper* are related.

In addition to the behavioral mutants isolated in our high-throughput screens, we identified a mutant line with apparent progressive neurodegeneration. These animals are phenotypically normal at birth, but exhibit loss of motor coordination and a concomitant decrease in activity by 10 weeks of age, with females affected earlier and with greater severity than males. Preliminary histological characterization suggests that this is due to neurodegeneration in several cortical, subcortical and brainstem regions.

We are currently attempting to identify the causative mutations in these lines using a number of mapping strategies, including analysis of both intercross and backcross progeny and the use of closely-related strains to limit background QTL effects. We will present our progress on these mapping efforts as well as on the further phenotypic characterization of these mutant lines.

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DOMESTICUS MEETS MUSCULUS: PHENOTYPIC DIVERSITY IN THE NEW C57B6/J CHR^{PWD} CONSOMIC STRAIN SETKL Svenson¹, S Petkova¹, B Paigen¹, J Forejt²¹The Jackson Laboratory, Bar Harbor, Maine, United States, ²Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Diversity is the spice of life. A broad spectrum of inbred mouse strains offers a powerful resource for studying genotypic and phenotypic diversity in biological traits. Divergent genomes can be further interrogated by mixing them to generate new resources such as recombinant inbred or consomic (chromosome substitution) strain sets. Use of these strains has yielded high returns on research investments for understanding functional genomics. Strain PWD/PhJ is pure *Mus mus musculus* and separated from *Mus mus domesticus* more recently than other *Mus* sub-species such as *spretus*, *castaneus* or *mollosinus*, and hence have better breeding success with *domesticus* derived strains. Compared to *domesticus*, PWD offers a high degree of sequence variation and also shows significant phenotypic differences, including plasma cholesterol and glucose levels, body composition, bone mineral density, coagulation factors and many hematological parameters (peripheral blood leukocytes, monocytes, granulocytes, hematocrit). Chromosome substitution strains have recently been completed in which each strain carries a single chromosome from PWD on a C57BL/6J background. We are conducting comprehensive phenotyping of each of these strains as they become available. Phenotypic characterization of 12 of these strains has localized many of these differences to specific chromosomes. Many of the parent strain differences are reflected in the consomic strains and some strains have phenotypes unlike either parent. Our results support the utility of chromosome substitution strain sets for rapidly localizing phenotypic diversity among inbred strains as well as use in refining QTLs and advancing our understanding of complex diseases.

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MBD3 IS REQUIRED FOR THE MAINTENANCE OF PATERNAL METHYLATION AND REPRESSION OF THE IMPRINTED H19 LOCUS

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Paternal repression of the imprinted gene *H19* is regulated by DNA elements that are shared with *Igf2*. These common DNA elements consist of a differentially methylated domain (DMD) that is methylated in the germline in a parental specific manner crucial for imprinted expression of both of these genes. *H19* is paternally methylated and maternally expressed; therefore expression of *H19* correlates with the methylation status of the DMD. The methyl-CpG binding (MBD) proteins are likely candidates to explain how these methylated domains are recognized and silenced.

To test for a possible role for MBDs in imprinting, we used RNAi to reduce both Mbd3 RNA and protein levels in blastocysts. In RNAi blastocysts, but not control embryos, paternal *H19* expression was detected in 25-40% of the embryos, supporting our hypothesis that Mbd3 is required for repression of *H19* on the paternal allele. Expression of *Snrpn* and *Gtl2*, two other imprinted genes, was not affected by Mbd3 reduction, suggesting these genes may employ a different Mbd for repression.

The *H19* DMD is methylated during germ cell maturation in males. After fertilization, the paternal genome undergoes demethylation. To test the hypothesis that Mbds may protect methylation at imprinted loci, RNAi blastocysts were subjected to bisulfite mutagenesis to examine the status of methylated CpGs in the absence of Mbd3. At the *H19* DMD, half of the paternal strands are partially or fully unmethylated when Mbd3 is reduced. Control embryos retain methylation on 85% of paternal strands, suggesting that Mbd3 plays an important role in the maintenance of methylation at the DMD as well as its role in repression of the paternal allele.

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A MOUSE MODEL OF ER-TO-GOLGI PROTEIN TRANSPORT DEFICIENCY

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Many diseases involve defects in intracellular protein trafficking. Combined deficiency of factors V and VIII (F5F8D) is an autosomal recessive disorder associated with simultaneous reduction of plasma FV and FVIII, and mild to moderate bleeding symptoms. By positional cloning, we have identified mutations in two genes (*LMAN1* and *MCFD2*) that are the causes for the disorder. We showed that *LMAN1* and *MCFD2* form a stable complex localized to the early secretory pathway of the cell. Study of all available patients indicated that mutations in *LMAN1* and *MCFD2* may account for all cases of F5F8D. We hypothesize that *LMAN1* binds *MCFD2* to form a cargo receptor complex required for efficient ER-to-Golgi transport of FV and FVIII. To further study the cargo receptor function of *LMAN1* *in vivo*, we generated mice deficient in *LMAN1*. Homozygous null mice exhibit reduced levels of both FV and FVIII in plasma, but normal level of FV in platelets, with no other obvious abnormalities. Matings of heterozygous mice on a mixed genetic background yielded offspring of the expected Mendelian genotype distribution. However, a backcross into the C57BL/6J background revealed an unexpected partial perinatal lethality, suggesting that at least in mice, *LMAN1* has additional functions. Analysis of the embryonic fibroblasts detected no general defect in COPII-coated vesicle formation resulting from *LMAN1* deficiency. To our knowledge, *LMAN1* knockout mice provide the first mammalian animal model of ER-to-Golgi protein transport deficiency. Ongoing studies are investigating the basis for the strain-specific perinatal lethality and using these animals to explore the role of *LMAN1* in the intracellular trafficking of FVIII.