

**MODELING DISEASE BY GENOME MANIPULATION/MUTAGENESIS  
ORAL PRESENTATION****TUESDAY NOVEMBER, 14****2.00PM - 2.15PM****O22****RWHS IS A MOUSE MODEL FOR BOCHDALEK CONGENITAL DIAPHRAGMATIC HERNIA IN HUMANS**AW Hart<sup>1</sup>, JE Morgan<sup>1</sup>, B Doe<sup>1</sup>, K West<sup>1</sup>, L McKie<sup>1</sup>, J Schneider<sup>2</sup>, S Bhattacharya<sup>2</sup>, IJ Jackson<sup>1</sup>, SH Cross<sup>1</sup><sup>1</sup>MRC Human Genetics Unit, Edinburgh, Scotland, United Kingdom, <sup>2</sup>Wellcome Trust Centre for Human Genetics, Oxford, England, United Kingdom

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, *Rwhs* causes white spots on the retina when heterozygous and is recessive lethal. High-throughput magnetic resonance imaging of embryos showed that *Rwhs* homozygotes have pulmonary hypoplasia and Bochdalek congenital diaphragmatic hernia. This malformation is present in 1/2500 live human births, and even with corrective surgery has a mortality of ~30%. Currently, there is no mouse model for this condition. We mapped *Rwhs* to a small interval on chromosome 11 and sequenced the exons and splice junctions of all the genes present. We found one nucleotide substitution that caused a missense mutation in a novel ubiquitously-expressed gene *Tmem98* that is predicted to encode a transmembrane protein. This point mutation leads to the substitution of a highly-conserved isoleucine by threonine. We are taking two approaches to confirm that this mutation underlies the *Rwhs* phenotype. In the first, we identified a second mutant allele of *Tmem98* by screening an archive of parallel DNA and sperm samples from ENU-mutagenised mice and recovered mice carrying the mutation by ICSI. This second point mutation converts the initiating methionine to a leucine and is predicted to ablate function. Non-complementation testing is currently underway. The ability of a BAC containing *Tmem98* to rescue the *Rwhs* recessive lethal phenotype is also being tested. *Rwhs* is the first model for Bochdalek congenital diaphragmatic hernia and will be useful in elucidating the function of *Tmem98*.

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**ORAL PRESENTATION**

**TUESDAY NOVEMBER, 14 2.15PM - 2.30PM**

**O23**

**MAPPING AND CHARACTERIZATION OF NSE5, A NOVEL MOUSE DEAFNESS MUTATION**

FJ Probst<sup>1</sup>, A Xia<sup>1</sup>, AS Salinger<sup>1</sup>, N Hong<sup>2</sup>, HY Tang<sup>1</sup>, RL Alford<sup>1</sup>, JS Oghalai<sup>1</sup>, MJ Justice<sup>1</sup>

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The study of mouse deafness mutants has led to the cloning of a number of human hearing loss genes. Here, we report the mapping and characterization of a novel ENU-induced mouse deafness mutant, *nse5*. Homozygous mutants have no detectable auditory brainstem response to sounds up to 80 dB at frequencies ranging from 4 to 90 kHz, demonstrating severe-to-profound hearing impairment. While most mouse deafness mutants show circling and head-tossing behaviors in addition to hearing impairment, *nse5* homozygotes behave normally and have no noticeable vestibular defects at up to 6 months of age. The gross appearance of the external and middle ears of mutant animals is normal. Microscopic analysis of the cochleas of mutant animals reveals that homozygous animals have numerous vacuoles in the outer hair cells that are not seen in heterozygous controls. We hypothesize that these vacuoles may represent the defective transport of neurotransmitters in mutant animals, which drastically reduces their ability to transduce sound waves into nerve impulses. Genetic analysis of more than 100 backcross and intercross progeny has localized the mutant locus to a 1.2 Mb interval on mouse chromosome 15. A missense mutation has been found in a poorly-characterized candidate gene in this region. Transgenic rescue experiments are currently underway, as is the sequencing of the human homolog of this gene in a panel of humans with hearing impairment. Ongoing experiments will help to understand how this novel gene causes hearing impairment in mice.

**MODELING DISEASE BY GENOME MANIPULATION/MUTAGENESIS  
ORAL PRESENTATION****TUESDAY NOVEMBER, 14****2.30PM - 2.45PM****O24****A SENSITIZED MOUSE MUTAGENESIS SCREEN FOR NOVEL LOCI REGULATING MAMMALIAN NEURAL CREST DEVELOPMENT**

D Watkins-Chow, I Matera, D Silver, SK Loftus, D Larson, K Buac, C Rivas, E Elliot, WJ Pavan  
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A mouse mutagenesis program is in progress to screen for mutations disrupting mammalian neural crest cell development. The focused phenotype screen has been designed to detect mutations affecting neural crest derived melanocytes and peripheral nervous system cells. Progeny of N-ethyl-N-nitrosourea (ENU) treated mice are being bred to *Sox10<sup>LacZ/+</sup>* mice (Britsch et al.) carrying a disruption in a transcription factor important for neural crest cell development. These mice manifest subclinical neural crest defects due to haploinsufficiency for SOX10. The sensitized screen uncovers mutations that act synergistically with *Sox10<sup>LacZ/+</sup>* resulting in clinically visible phenotypes such as increased white coat color spotting. Additionally, third generation embryos are being generated in a backcross to screen for embryonic phenotypes that alter expression of the LacZ reporter gene in *Sox10* expressing cells. Our 3-generation breeding strategy utilizes two different mouse strains, BALB/cJ and C57BL/6J, to facilitate mapping of both dominant and recessive phenotypes and allows for subsequent recovery of lethal phenotypes. To date, we have identified three heritable phenotypes from the dominant screen of over 150 pedigrees and five heritable mutations from a recessive embryonic screen of 100 pedigrees. The phenotypes observed in the embryonic screen include ectopic expression of Sox10-LacZ, abnormal cell patterning and migration, and loss of Sox10-LacZ expression in subsets of peripheral nervous system cell lineages. None of the loci localize to genes for the major mouse spotting mutants, demonstrating the feasibility of this approach for identifying novel loci regulating neural crest development.

**MODELING DISEASE BY GENOME MANIPULATION/MUTAGENESIS**

**ORAL PRESENTATION**

**TUESDAY NOVEMBER, 14**

**2.45PM - 3.00PM**

**O25**

**IDENTIFICATION OF GENES REQUIRED FOR NORMAL FOREBRAIN DEVELOPMENT USING ENU MUTAGENESIS**

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The forebrain is the largest portion of the human brain and is responsible for higher order cognitive functions, including reasoning and memory. Despite its central importance, many of the genes required for normal cortical development remain unknown. We have begun an ENU mutagenesis screen in the mouse to identify genes required for forebrain development. We use a 3-generation breeding strategy to obtain autosomal recessive mutations and observe for phenotypes at E18.5. This stage of development, just before birth, allows the embryo to survive with severe defects in organogenesis that would be lethal in a newborn animal. These should resemble birth defects seen in the human newborn population and provide animal models of clinical significance. Indeed, the utility of this approach has been validated by its application for the discovery of a causal gene for congenital diaphragmatic hernia (Ackerman et al., PLoS Genetics, 2005). Our approach is designed to be unbiased, which should serve to uncover novel or poorly annotated genes with a role in nervous system development, as well as implicate genes in brain development which have been previously characterized in other physiological settings. Our neurodevelopmental screen has three components: a) a morphologic examination complemented by histological analysis, b) use of a RARE-lacZ reporter allele to highlight distinct brain structures, and c) use of *Lis1* as a sensitizing mutation. While the screen is still in progress, we have already identified multiple lines of interest. Some of the mutations affect multiple tissues in the embryo, while others appear to be specific to the brain. The most intriguing phenotype shows severe cortical agenesis as well as defects in the appendicular skeleton. Other mutations show significant neuronal heterotopias or changes in brain size. Several phenotypes appear normal upon gross examination and are only detectable upon histological analysis. One line highlights the utility of a reporter allele as the phenotype is only revealed by the perturbation of its expression pattern in the cortex. Several mutants have been mapped using a whole-genome SNP genotyping panel (Moran et al., Genome Research 2006) and positional cloning is in progress.

**MODELING DISEASE BY GENOME MANIPULATION/MUTAGENESIS  
ORAL PRESENTATION**

**TUESDAY NOVEMBER, 14**

**3.00PM - 3.15PM**

**O26**

**MOUSE MODELS OF OTITIS MEDIA, MIDDLE EAR INFLAMMATORY DISEASE – THE JEFF MUTANT CARRIES A MUTATION IN AN F-BOX GENE**

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Otitis media (OM), inflammation of the middle ear, is the most common cause of hearing impairment and surgery in children. Recurrent (ROM) and chronic (COME) forms of otitis media are known to have a strong genetic component, but nothing is known of the underlying genes involved in the human population. From a deafness screen as part of the Harwell ENU mutagenesis programme we have identified two novel dominant mouse mutants, *Jeff* and *Junbo*, which develop a conductive deafness due to a chronic suppurative otitis media. Both these mutants represent models for chronic forms of middle ear inflammatory disease in humans. The *Jeff* mutant carries a mutation in an F-box gene, *Fbxo11*, a member of a large family of proteins that are specificity factors for the SCF E3 ubiquitin ligase complex. *Fbxo11* is expressed in the mucin secreting cells of the middle ear epithelia during the period at which otitis media develops in *Jeff*. *Jeff* homozygotes show cleft palate, facial clefting and perinatal lethality and *Fbxo11* is also expressed in the epithelial palatal shelves during development. Initial studies of *FBXO11* SNPs in human OM families have uncovered nominal evidence of association, indicating the genetic involvement of human *FBXO11* in chronic otitis media with effusion and recurrent otitis media (Segade et al., 2006 in press). *Fbxo11* is one of the first molecules to be identified contributing to the genetic etiology of otitis media.