

**Wednesday November, 15**  
**10.30am – 12.30pm**  
**Poster Session 4**  
**Development/Aging**  
**Posters P90 – P99**

- P90 CRANIOFACIAL AND OTHER SKELETAL DEFECTS IN MUTANT MICE REVEAL SHARED AND GENE-SPECIFIC FUNCTIONS OF THE THREE  $G\alpha_1$  PROTEINS IN NEURAL CREST AND SOMITES**  
NW Plummer<sup>1</sup>, K Spicher<sup>2</sup>, L Birnbaumer<sup>1</sup>  
<sup>1</sup>NIH, National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States, <sup>2</sup>Heinrich Heine University, Duesseldorf, Germany
- P91 ESTABLISHING MULTIPLE MOUSE LINES POSSESSING POINT MUTATION ON BETA-CATENIN (CTNNB1) GENE**  
T Murata, K Karouji, Y Sakuraba, R Fukumura, H Kaneda, S Wakana, T Noda, T Shiroishi, Y Gondo  
RIKEN Genomic Sciences Center, Yokohama, Japan
- P92 CREATING AND CHARACTERIZING A MURINE MODEL OF TREACHER COLLINS SYNDROME (TCS)**  
L Li, R Shiang  
Virginia Commonwealth University, Richmond, VA, United States
- P93 APOPTOSIS AS A MECHANISM FOR CAUDAL TRUNCATION IN ADRENOCORTICAL DYSPLASIA MICE**  
AS Krause, MJ Morley, T Else, BC O'Connor, GD Hammer, DO Ferguson, CE Keegan  
University of Michigan, Ann Arbor, MI, United States
- P94 MAPPING OF THE WAVED WITH OPEN EYES (WOE) LOCUS**  
D Sidjanin, E Hassemer, L Jackson, E Talamas, B Chang  
<sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States, <sup>2</sup>The Jackson Laboratory, Bar Harbor, ME, United States
- P95 A MOUSE MODEL FOR JUVENILE HYDROCEPHALUS**  
EA Glick, J Ramalie, E Steshina, JV Schmidt  
University of Illinois at Chicago, Chicago, IL, United States
- P96 THE ROLE OF AKT IN MOUSE**  
W Chen<sup>1</sup>, K Coleman<sup>2</sup>, N Hay<sup>1</sup>  
<sup>1</sup>University of Illinois at Chicago, Chicago, IL, United States, <sup>2</sup>Pfizer Global Research and Development, Groton, United States
- P97 HYBRID EPIGENETIC MISREGULATION IS ASSOCIATED WITH DEVELOPMENTAL DEFECTS IN PROLIFERATION AND DIFFERENTIATION**  
PB Vrana<sup>1</sup>, AR Duselis<sup>1</sup>, QK Nguyen<sup>1</sup>, RJ O'Neill<sup>2</sup>, MJ O'Neill<sup>2</sup>, JA Mack<sup>2</sup>, C Obergfell<sup>2</sup>  
<sup>1</sup>University of California Irvine, Irvine CA, United States, <sup>2</sup>University of Connecticut, Storrs, CT, United States
- P98 RETROTRANSPOSONS IN MOUSE OOCYTES AND CLEAVAGE-STAGE EMBRYOS**  
KW Hutchison<sup>1</sup>, AE Peaston<sup>2</sup>, BB Knowles<sup>2</sup>  
<sup>1</sup>The University of Maine, Orono, ME, United States, <sup>2</sup>The Jackson Laboratory, Bar Harbor, ME, United States
- P99 TRANSCRIPTIONAL REGULATION OF EPIDERMAL BARRIER DEVELOPMENT**  
JA Segre, S Patel, C Strong  
NHGRI, Bethesda, MD, United States

**P90****CRANIOFACIAL AND OTHER SKELETAL DEFECTS IN MUTANT MICE REVEAL SHARED AND GENE-SPECIFIC FUNCTIONS OF THE THREE  $G\alpha_i$  PROTEINS IN NEURAL CREST AND SOMITES**

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The human and mouse genomes each contain 16 paralogous G protein alpha subunit genes. Within this gene family, the “inhibitory” alpha subunits ( $G\alpha_i$ ) are encoded by three closely related paralogs: *Gnai1*, *Gnai2*, and *Gnai3*. The sequence similarity of these three genes suggests that they may have overlapping functions. We report that inbred 129/SvEv mice homozygous for loss of *Gnai3* have abnormal pigmentation on the snout, frontal hematomas, and enlarged interfrontal bones consistent with a role for the gene in the cranial neural crest. The mice also have fusions of ribs and lumbar vertebrae indicating a requirement for *Gnai3* in somites. Mice lacking *Gnai1* or *Gnai2* have neither craniofacial nor skeletal defects. Loss of both *Gnai3* and *Gnai1* or *Gnai3* and *Gnai2* results in more severe rib fusions but has no effect on lumbar fusions or the craniofacial defects. Normal myotome morphology in *Gnai3/Gnai1* double knockout mouse embryos suggests that  $G\alpha_i$  is specifically required for signal transduction in the sclerotome. Penetrance and expressivity of the pigment defect, rib fusions, and lumbar fusions are altered in the F2 generation of a C57BL/6 x 129/SvEv intercross. Thus, the mutant mice reveal a gene-specific function of *Gnai3* in neural crest, genetic interaction of the three  $G\alpha_i$  genes in somites, and the ability of strain-specific alleles of other, unknown genes to modify the phenotypes.

**P91****ESTABLISHING MULTIPLE MOUSE LINES POSSESSING POINT MUTATION ON BETA-CATENIN (CTNNB1) GENE**

T Murata, K Karouji, Y Sakuraba, R Fukumura, H Kaneda, S Wakana, T Noda, T Shiroishi, Y Gondo  
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Beta-catenin is a very unique protein, regarding two different aspects. One is a structural protein, bridging the cytoplasmic domain of a cell-cell interacting protein, cadherin and cytoskeletal actin together with alpha-catenin. The other is a signaling molecule, locating at the bottom of the canonical Wnt pathway, regulating cell fate determination or cell proliferation through transcriptional activation. The null mutant mice are known to be embryonic lethal at an early stage. More mutant alleles including point mutations and conditional knockouts are thus needed to elucidate the beta-catenin function at later stages.

We have been presenting the “ENU-based gene-driven” method to identify point mutations in specific genes from the genome archive originated from about eight thousand G1 male mice produced by ENU mutagenesis. By this method, we have been screening point mutations on the *beta-catenin* (*ctnnb1*) gene. Seven mutations have been found by screening about one forth of the archive. One is intronic and the others are exonic (3 missense, 1 nonsense, and 2 synonymous). Three missense mutations cause drastic amino acid substitution from polar to non-polar amino acid residue, or vice versa. Nonsense mutation codes truncated protein lacking C-terminal transactivation domain, or may cause null mutation resulted from nonsense-mediated decay. These four mutant lines, except for two synonymous, were worthwhile to analyze further, and all of them were successfully retrieved as live mice by IVF from the frozen-sperm archive.

**P92****CREATING AND CHARACTERIZING A MURINE MODEL OF TREACHER COLLINS SYNDROME (TCS)**L Li, [R Shiang](#)

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Treacher Collins syndrome (TCS) is an autosomal dominant craniofacial developmental disorder and is caused by mutations in the *TCOF1* gene. The tissues affected in TCS arise from the first and second branchial arches. Heterozygous *Tcof1* knockout mice have severe craniofacial abnormalities overlapping with TCS patients. However, these mice are neonatal lethal, which circumvent their further analysis. In this study, we generated a *Tcof1* conditional allele with loxP sites flanking exon 1. These mice were crossed with *Wnt1-cre* transgenic mice to generate a conditional knock out of *Tcof1* specifically in neural crest (NC) cells. Surprisingly, unlike the conventional heterozygous knockout mice, conditional heterozygous knockout mice are phenotypically normal, which indicates that *Tcof1* functions in tissues in addition to NC cells during development. Homozygous conditional knockout mice show craniofacial abnormalities resembling TCS patients including defects in maxillary, mandible and external ears. Embryonic lethality at ~ E12.5 of homozygous knockout embryos also suggests additional functions of *Tcof1* for embryo survival other than craniofacial development. Alcian blue cartilage staining shows that the absence of the first and second branchial arch cartilages, the Meckel's and Reichert's cartilages. Previously, transcription factor *Cnbp* was identified as *Tcof1* downstream gene in a neurabloma cell line study. *Cnbp* is known to affect cell growth and important for craniofacial development. Whole mount *in situ* hybridization shows that *Cnbp* expression is decreased in a proportion of the homozygous conditional knockout mouse embryos. Our results suggest that *Tcof1* may affect craniofacial development through *Cnbp*.

**P93****APOPTOSIS AS A MECHANISM FOR CAUDAL TRUNCATION IN ADRENOCORTICAL DYSPLASIA MICE**AS Krause, MJ Morley, T Else, BC O'Connor, GD Hammer, DO Ferguson, [CE Keegan](#)

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Adrenocortical dysplasia (*acd*) is a spontaneous autosomal recessive mouse mutant with developmental defects in organs derived from the urogenital ridge. In addition, the *acd* mutation exhibits embryonic lethality on certain genetic strains, and analysis of *acd* mutant embryos reveals a striking caudal truncation phenotype. We have previously characterized the *acd* mutation as a splicing defect in a gene (*Acad*), which encodes a novel component of the telomere cap complex that functions to maintain telomere integrity. Based on the proposed function of ACD as a telomeric protein, we hypothesized that the loss of cells in the caudal region might be due to telomere dysfunction, leading to apoptosis or cell cycle arrest via activation of p53. To further explore the relationship between the *acd* mutation and caudal dysgenesis, we investigated potential mechanisms leading to caudal truncation in *acd* mutant embryos. We observed an increased number of chromosomal end-to-end fusions in metaphase karyotypes from *acd* MEFs compared to wildtype cells, consistent with the presence of telomere dysfunction. We also observed a significant increase in the number of apoptotic cells in the caudal region of *acd* embryos, but no gross differences in proliferation. These studies confirm the presence of telomere dysfunction in *acd* MEF cells and suggest that apoptosis is the predominant mechanism leading to caudal truncation in *acd* mice. We are currently crossing *acd* mice to p53 null mice to determine whether p53 deficiency rescues the caudal dysgenesis phenotype. This work will shed important insights on the role of telomere stability during development.

**P94****MAPPING OF THE WAVED WITH OPEN EYES (WOE) LOCUS**

D Sidjanin, E Hassemer, L Jackson, E Talamas, B Chang

<sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States, <sup>2</sup>The Jackson Laboratory, Bar Harbor, ME, United States

Waved with open eyes (*woe*) is an autosomal recessive mouse mutant that arose spontaneously on the C57B6/DBA2-F1 background. Phenotypically, the mouse shows a wavy coat, microphthalmia, and enlarged heart and oesophagus. The goal of this study was to identify the mutation responsible for the *woe* phenotype. The *woe* mice were outcrossed to C3H and F1 mice were backcrossed to *woe*. We generated 102 F2 backcross progeny which were phenotypically evaluated at three weeks of age. Genome wide scan of the backcross progeny identified linkage between the *woe* locus and microsatellite markers on the proximal arm of mouse chromosome 12. No recombinants were identified between the *woe* phenotype and *D12Mit12*. Evaluation of the *woe* critical region identified A Disintegrin and Metalloproteinase Domain 17 (*Adam17*) as a candidate gene. In humans, *ADAM17* has been associated with ectodermal shedding of TNF- $\alpha$  and the proteolytic release of several other cell-surface proteins, including p75 TNF-receptor, interleukin 1 receptor type II, p55 TNF-receptor, TGF- $\alpha$ , L-selectin, growth hormone receptor and the amyloid precursor protein. The sequence analysis of *Adam17* in *woe* revealed a 1 bp substitution in exon 7. This C794T substitution leads to a Thr265Met amino acid change in the metalloproteinase domain of *Adam17*. Thr265 is evolutionary highly conserved residue and may be essential for the proper metalloprotease function of *Adam17*. Currently, we are investigating the effect of the Thr265Met substitution on the metalloprotease catalytic function of *Adam17* and the *woe* phenotype.

**P95****A MOUSE MODEL FOR JUVENILE HYDROCEPHALUS**

EA Glick, J Ramalie, E Steshina, JV Schmidt

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Juvenile hydrocephalus, the accumulation of fluid in the ventricles of the brain, is a cause of significant morbidity among human children, but potential genetic causes of this disease are unknown. To identify enhancers of an unrelated gene, transgenic mice were generated carrying a short genomic region fused to the *lacZ* reporter. When analyzed for *lacZ* expression, however, embryos from multiple transgenic lines showed entirely different staining patterns. These data indicate that *lacZ* is regulated by elements present at the unique integration site for each transgenic line, and that no strong enhancers are present in the transgene itself. Such inadvertent "enhancer-trap" vectors are useful tools for gene identification.

One transgenic line showed *lacZ* staining at e12.5 in the epiphysis of the diencephalon, the rudiment of the pineal gland, and subsequently in the habenular nuclei and regions of the hypothalamus. When homozygous transgenic animals were generated, ~80% of the mice developed severe hydrocephalus between 2 and 6 weeks of age. These phenotypes suggest that the transgene disrupted expression of a gene involved in normal brain development.

The integration site of the transgene was mapped to mouse chromosome 9, and candidate genes are being analyzed for altered expression in the mutant mice. Analysis of these animals is likely to identify a novel gene involved in fluid regulation within the developing brain, a gene that may also be involved in human hydrocephalus. Additionally, this gene may function in the development of the pineal gland, an important neuroendocrine organ that is poorly understood.

**P96****THE ROLE OF AKT IN MOUSE**

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Akt, also known as Protein Kinase B (PKB), is a serine/threonine kinase. There are three isoforms of Akt in mammals: Akt1 (PKB $\alpha$ ), Akt2 (PKB $\beta$ ), and Akt3 (PKB $\gamma$ ), which are encoded by three separate genes. Akt signalling has been well studied and it belongs to the PI3-K pathway. In order to understand the role of Akt *in vivo*, we have generated Akt1, Akt2 and Akt3 knockout mice. Each individual Akt1 knockout mouse shows a very mild abnormal phenotype, indicating a redundancy of Akt. Therefore, we have crossed individual Akt knockout mouse to generate different double and triple knockout mice, and some of the knockout mice show interesting phenotypes. We will discuss some of the phenotype of those compound knockout mice.

**P97****HYBRID EPIGENETIC MISREGULATION IS ASSOCIATED WITH DEVELOPMENTAL DEFECTS IN PROLIFERATION AND DIFFERENTIATION**

PB Vrana<sup>1</sup>, AR Duselis<sup>1</sup>, QK Nguyen<sup>1</sup>, RJ O'Neill<sup>2</sup>, MJ O'Neill<sup>2</sup>, JA Mack<sup>2</sup>, C Obergfell<sup>2</sup>

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Crosses between two species of the rodent genus *Peromyscus* have been shown to produce parent-of-origin specific growth and developmental defects. The hybrid defects are particularly pronounced in the placenta. *P. maniculatus* (strain - BW) females mated to *P. polionotus* (strain - PO) males produce placentae half the size of the parental species, as well as growth-retarded embryos. In contrast, PO females mated to BW males result in embryonic and placental overgrowth as well as deleterious phenotypes. These parent – of – origin dependent phenotypes suggested perturbations of genomic imprinting. Imprinted genes display biased allelic expression dependent on parental origin, and many are involved in growth regulation. Previous studies of the hybrids have shown altered expression of imprinted genes, and genetic linkage to imprinted domains.

We took a broad assessment of hybrid placental gene perturbations by utilizing *Mus musculus* cDNA microarrays. In verifying the array data, genes influencing cell-cycle and extra-cellular matrix (ECM) were prominent. We then undertook assays to demonstrate differences in apoptosis, differentiation and proliferation at three time points. Genes regulating the G1/S phase checkpoint are particularly affected. ECM genes are downstream targets of cell cycle regulation, and their misregulation is consistent with many of the dysmorphic phenotypes. Both hybrids display developmental shifts relative to the parental strains. This shifting of regulation has led to an increase in proliferation, decrease in differentiation and lack of patterning in the hypertrophic placentae. The growth-retarded hybrids display a general lack of proliferation and stem cell progenitors.

**P98****RETROTRANSPOSONS IN MOUSE OOCYTES AND CLEAVAGE-STAGE EMBRYOS**KW Hutchison<sup>1</sup>, AE Peaston<sup>2</sup>, BB Knowles<sup>2</sup><sup>1</sup>The University of Maine, Orono, ME, United States, <sup>2</sup>The Jackson Laboratory, Bar Harbor, ME, United States

The events of very early mammalian development depend on the union of the genomes of the oocyte and sperm to form the genome of the totipotent embryo. This necessitates extensive changes in genomic function, usually described as genomic reprogramming, and generally understood to be mediated by serial epigenetic modifications of nuclear DNA. We observed sequential, developmentally regulated activation and silencing of retrotransposons, in particular Class III endogenous retroviral (ERV) elements, in mouse oocytes and preimplantation embryos. Additionally, retrotransposons acted as alternative promoters and first exons for diverse genes, synchronising their expression. Our working hypothesis is that differential transposable element expression triggers sequential reprogramming of the embryonic genome during the oocyte-to-embryo transition and in preimplantation embryos. Endogenous retroviruses are usually epigenetically silenced, and marked by cytosine methylation of CpG dinucleotides in their long terminal repeat (LTR) promoters. So their activation and silencing indicates underlying epigenetic change. In ongoing work, we are investigating the methylation status of individual unique genomic ERV loci to determine whether DNA methylation status is correlated with expression data. In addition we are using computational and experimental approaches to determine whether full-length ERVs are expressed from multiple dispersed loci or only from a specific subset. Our results address questions regarding changes in chromatin structure in the early embryo.

**P99****TRANSCRIPTIONAL REGULATION OF EPIDERMAL BARRIER DEVELOPMENT**JA Segre, S Patel, C Strong

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The skin barrier serves two essential functions for survival by preventing both the escape of moisture and the entry of infectious or toxic substances. Clinical advances in the last 100 years, including the discovery of antibiotics, emollients and semi-permeable sterile dressings, have reduced the risk of a life-threatening outcome due to an impaired barrier. However, our bodies still elicit a strong genomic response to this stress, programmed to consider the possibility of massive water loss or a septic bacterial infection. Here, we study KLF4 and GATA-3, two transcription factors that regulate the process of barrier acquisition. We dissect out the genetic programs that they regulate by identifying their downstream targets with microarrays. We assess whether these are direct targets with biochemical strategies, including reporter assays and chromatin immunoprecipitation, in conserved noncoding sequences. GATA-3 and KLF4 regulate distinct pathways required to establish the epidermal barrier. However, in both cases the impaired barrier evokes a “biosensor” compensatory response from effectors of the innate immune system. These investigations shed light on the common skin disorders, psoriasis and atopic dermatitis, which exhibit decreased barrier function and specific immune responses.

