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FULL ABSTRACTS
Species composition of the gut microbiota in murine models; a complex polygenic trait. Andrew K. Benson. Food Science and Technology, University of Nebraska, Lincoln, NE.

The vertebrate GI tract is home to trillions of microbes comprising hundreds to thousands of species that carry out diverse metabolic functions and play crucial roles in development of the immune system. Numerically dominant microbial species in the vertebrate gut belong to a small number of bacterial phyla, but the specific taxa and the relative ratios of dominant taxa vary tremendously across individuals. Abnormalities in the ratios of the dominant phyla (dysbiosis) are associated with many complex lifestyle diseases such as obesity, and inflammatory bowel disease in humans. To identify factors which predispose individuals to dysbiosis, we use quantitative genetic analysis in murine models to elicit factors that influence species abundances in the gut microbiota. Using quantitative pyrosequencing to phenotype the microbiota, we have observed that a small number of bacterial taxonomic groups, collectively referred to as the Core Measurable Microbiota (CMM), are consistently measurable and log-normally distributed across individuals. QTL analysis of the CMM in an advanced intercross line (AIL) at generation four identified 13 QTL associated with the relative abundance of 26 taxonomic groups of the CMM. At least one taxon in each dominant microbial phylum is affected by QTL; thus, the effects of host genetic factors occur across the broad phylogenetic space of the microbiota and are likely to provide a framework for assembly of the microbiota early in life. Further support for this framework concept comes from the observation that some of the QTL are pleiotropic, controlling groups of apparently unrelated organisms that behave like correlated phenotypes. Ongoing mapping studies of CMM from the same AIL (now at generation ten) and additional mapping populations continue to implicate some of the same QTL and several new QTL and they provide evidence for gene X diet interactions in control of the microbiome. Collectively, these studies characterize the gut microbiome as a polygenic trait influenced by complex interactions of environmental and host genetic factors.
1 A cell-based screen for genes involved in mammalian cilia formation and function. Emma A. Hall, Pleasantsine Mill, Ian J. Jackson. MRC Human Genetics Unit, Edinburgh, Scotland, United Kingdom.

Primary cilia are essential for mammalian development, and are important for many signalling events, particularly Hedgehog (Hh) signalling. Mutations for cilia genes show perturbed Hh responses. We developed a cell-based RNAi screen to identify new genes involved in cilia formation and/or function. We are screening candidate genes identified by complementary analysis plus proteomic and transcriptomic studies. The screen provides two readouts: an image-based readout identifies genes required for cilia formation, assayed by high-throughput immunofluorescence microscopy. A second, functional, readout measures Hh responsiveness, for which cilia are necessary. The screen has revealed several candidate genes, which may have a role in ciliogenesis. We have performed an in depth analysis of one such candidate gene, for which we have generated a gene trap mouse line. Identification of novel ciliogenic genes will aid the analysis of diverse functions of primary cilia in development and help explain the varied phenotypes seen in human ciliary diseases, or ciliopathies.

2 A Forward Genetic approach to identify Salmonella Typhimurium susceptibility loci using N-ethyl-N-nitrosourea (ENU) mutagenesis in mice. Megan M. Evi1, Kyoko E. Yuki1, Shauna M. Duphorne2, Etienne Richer2, Silvia Vidal1,2,4, Danielle Mao3,4. 1) Departments of Human Genetics, 2) Medicine; 3) Microbiology and Immunology, McGill University, Montreal, QC, Canada; 4) Complex Trait Group of the McGill Life Sciences Complex; 5) BioMedICin Consultants inc., Montreal, QC, Canada.

Salmonella, a Gram negative intracellular bacterium is a food-borne microorganism that infects a broad-spectrum of hosts. Acute systemic Salmonella Typhimurium infection in mice is recognized as an experimental model to study the pathogenesis of typhoid fever in humans. Our laboratory initiated a broad ENU (ubiquitin specific peptidase 18) mediated by specific components in the diet, rather than obesity or related metabolic effects. These dietary studies have important implications for both prevention and non-invasive treatment of intestinal cancer in humans.

5 Fine-Mapping QTL and Inferring Causing Pathways that Underlie Sixtine Murine Phenotypes. Jon Krohn1, Felix Agakigab2, Paul McKeigue3, Jonathan Flint1. 1) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom; 2) Centre for Public Health Sciences, University of Edinburgh, Edinburgh EH9 9AD, United Kingdom.

Following the crossing of inbred lines, the identification of quantitative trait genes (QTGs) is complicated by linkage disequilibrium, which produces quantitative trait loci (QTL) that contain low or high amounts of specific fats from coconut (Coco), corn (Corn), or olive (Olive) oils. Three outcomes were possible: (1) If obesity is crucial in polyp formation, all obese strains should develop more polyps, (2) If fat content is important, all strains fed a HFD should have increased polyp numbers, regardless of susceptibility phenotype, and (3) If obesity is not crucial, all obese strains should develop more polyps, and all strains fed a HFD should have increased polyp numbers. We screened three calorically balanced diets that contained low or high amounts of specific fats from coconut, corn, or olive. Three outcomes were possible: (1) If obesity is crucial in polyp formation, all obese strains should develop more polyps, (2) If fat content is important, all strains fed a HFD should have increased polyp numbers, regardless of susceptibility phenotype, and (3) If obesity is not crucial, all obese strains should develop more polyps, and all strains fed a HFD should have increased polyp numbers. We screened three calorically balanced diets that contained low or high amounts of specific fats from coconut, corn, or olive. Three outcomes were possible: (1) If obesity is crucial in polyp formation, all obese strains should develop more polyps, (2) If fat content is important, all strains fed a HFD should have increased polyp numbers, regardless of susceptibility phenotype, and (3) If obesity is not crucial, all obese strains should develop more polyps, and all strains fed a HFD should have increased polyp numbers.

6 Vacularized lens (vl): a multicistronic mouse mutant model of Neural Tube Defects (NTDs). Bo Li1,2, Paul G. Matteson1, Alejandro Q. Naito, Jr.2, Tara C. Matise1, James H. Millen3,2,1, 1) Center for Advanced Biotechnology and Medicine, Piscataway, NJ; 2) Neuroscience and cell biology, University of Medicine and Dentistry of New Jersey, Piscataway, NJ; 3) Department of Genetics, Rutgers University, Piscataway, NJ.

To understand the causes of mammalian NTDs, we are studying the vacularized lens (vl) mutation that arose spontaneously on the C3H/HeSnJ background. The mutation affects apposition/fusion of the neural folds, which leads to NTDs, lethality and abnormal pigmentation. The vl/phenotypes are due to a mutation in an orphan G-protein coupled receptor (Gpr161), which is expressed in the neural crest. Crossing vl mouse to the Molieu strain rescues the v defects. QTL analysis mapped three modifiers (Modvl1, Modvl2, Modvl3). One QTL being studied is Modvl3 (Lod=5.0, Chr18). Modvl3 is a Congenital anomaly embryos at different development stages were genotyped and vl-associated lethality was determined to occur between E8.5 and E9.5. Modvl3 is sufficient to rescue the lethality
The role of meiotic sex chromosome inactivation in sterility of interspecific hybrids. Tammy Bhattacharya1, Ondra Mihola1, Peter Šimek1, Mary Ann Handel1, Jiří Forajt1.

1) Mouse Molecular Genetics, Institute of Molecular Genetics, Prague, Czech Republic; 2) The Jackson Laboratory, Bar Harbor, ME, USA.

Hybrid sterility limits gene flow between related species. Two major hybrid sterility loci genes, Hstl/Prdm9 on Chr 17 and Hstx1 on X chromosome (Chr X), have been identified in sterile males from crosses between PWD/Ph and C57BL/6J (B6) strains derived from the Mus musculus domesticus subspecies. Here we focused on the molecular phenotyping of spermatogenic cells and the role of meiotic sex chromosome inactivation (MSCI) in hybrid sterility. FACS-sorted spermatocytes of (PWD X B6) F1 sterile males revealed meiotic block at mid-late pachynema and the TUNEL assay showed apoptosis of arrested spermatocytes. Around 85 % of histone H1-positive mid-pachynemas showed incomplete autosomal synapsis visualized by anti SYCP1 and SYCP3 antibodies. The unsynapsed chromosomes were decorated by ATR, RAD51 and DMC1, indicating the persistence of unrepaired double-strand breaks (DSB). Distribution of H3K9-dimethyl immunostaining pointed to meiotic silencing of unsynapsed chromatin (MSC1), a picture complemented by Cot-1 FISH on spermatocyte spreads. The same cells from sterile hybrids displayed phosphorylated histone H2AFX over the unsynapsed autosomes and disrupted XY-body. Fifty-five percent of mid-pachynemas in sterile males with XY-body still discernible had this sex chromosome compartment disrupted by intrusion of autosomal chromatin, while only 2 % cells with such anomaly were observed in controls. Only 20 % of pachynemas displayed normal frequency of MLH1 foci (>20 foci) detecting meiotic recombination in sterile hybrids compared to 62 % in controls. The microarray expression profiling of sterile testes revealed dysregulation of the X-linked genes. The RNA FISH of X-linked genes confirmed the failure of MSCI in mid-pachynemas of sterile males. It is proposed that a cascade of events, starting by the occurrence of unrepaired DSB in unsynapsed chromosomes, leads to disruption of MSCI, which acts as a male-specific meiotic checkpoint.

Fuz, a Planar Cell Polarity Gene Coordinates Signaling Pathways During Craniofacial Development. Zichao Zhang1, Bogdan Wlodarczyk2, Karen Niederreither1, Shankar Venkataraman1. 1) Department of Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA; 2) School of Integrative Biology, Philadelphia, PA.

We studied craniofacial development and the role of Fuz in craniofacial development using a conditional deletion of Fuz in Fuz+/− mice. Fuz expression was first apparent in the dorsal neural tube and later in ventral tissues and craniofacial regions during embryonic development coincident with craniofacial development. The Fuz−/− mice exhibit severe craniofacial deformities including anophthalmia, agenesis of the tongue and incisors, a hypoplastic mandible, cleft palate and hyperplastic mss-oriented Meckel’s cartilage. HH signaling is down-regulated in the Fuz null mice, while canonical WNT signaling is up-regulated revealing the antagonistic relationship of these two pathways. Meckel’s cartilage is expanded in the Fuz−/− mice due to increased cell proliferation associated with the up-regulation of WNT canonical target genes and decreased non-canonical pathway genes. Interestingly, cilia development was decreased in the mandible mesenchyme of Fuz null mice, suggesting that cilia may antagonize WNT signaling in this tissue. Furthermore, expression of Fuz decreased activation of WNT pathway genes as well as a WNT-dependent reporter. Finally, chromatin IP experiments demonstrate that β-catenin/TCP-binding directly regulates expression of Fuz. Conclusions: These data demonstrate a new model for coordination of HH and WNT signaling and reveal a FZD-dependent negative feedback loop controlling Wnt/β-catenin signalling.

A population-based model for mapping quantitative trait loci for susceptibility to environmentally-induced diseases. Michelle DeSimone1, Alan Bohn1, Garrison Glavich1, Katherine McCaffrey2, Heather Patison2, David Threadgill1. 1) Department of Genetics, NCSU, Raleigh, NC; 2) Department of Biology, NCSU, Raleigh, NC.

Exposure to environmental carcinogens through groundwater and dietary contamination occurs in many localities and is thought to result in tissue injury and disease in susceptible human populations. The interpretation of disease associations is under great debate in part due to the lack of an appropriate population-level experimental model with which to test the affect of inter-individual variation in xenobiotic metabolism, detoxification, and transport. To model the genetic heterogeneity of exposed human populations, we developed a unique interspecies population derived from FVB/N-Mdrba/1b−/−, a multi-drug resistant p-glycoprotein knockout mouse model, and CAST/EiJ a wild-derived strain that is a multi-drug resistant p-glycoprotein knockout mouse model, and CAST/EiJ a wild-derived strain that is. The imprinted Kcnq1 domain on mouse Chromosome 7 is under the control of a paternally expressed 90 kb non-coding RNA Kcnq1ot1, also known as Lit1. The syntenic human region on chromosome 11 is associated with Beckwith-Wiedemann fetal overgrowth syndrome and the cardiac disorder long QT syndrome. The majority of the genes within this 800kb domain are silenced on the paternal chromosome by Lit1. However, at least two genes escape silencing: Trpm5 (transient receptor potential protein 5) has biallelic expression and Kcnq1 (potassium voltage-gated channel, subfamily Q, member 1) has monoallelic expression in early development but switches to biallelic expression in fetal heart, liver and gut. We hypothesize that the genes that escape repression by Lit1 are under the control of regulatory elements and that the three-dimensional interactions between these elements allow the genes to be expressed on the paternal chromosome. We have confirmed that Kcnq1 undergoes a switch from mono- to biallelic expression during embryogenesis. Thus, we propose that enhancer activity is directing the biallelic expression of Kcnq1. Our bioinformatic analyses identified potential enhancer sequences. Some of these sequences have shown to exhibit occupancy of p300 during ChIP analysis. Also, these sequences showed enhancer activity in a dual luciferase assay. Further studies will be carried out in a model system in which mouse embryonic stem cells are differentiated into ventricular cardiomyocytes. This system recapitulates embryonic development and the mono- to biallelic switch. We hypothesize that Trpm5 escapes silencing in an independent chromatin domain. We identified and confirmed through ChIP analysis several binding sites for CTCF, the mammalian insulator protein, surrounding the Trpm5 promoter. The candidate CTCF binding sites have an in vivogenetic determinant.

Silencing by a Non-coding RNA is Overcome by Two Distinct Mechanisms. Lisa Korostowski, Natalie Sedlak, Nora Engel. Fels Institute for Cancer Research and Molecular Biology, Philadelphia, PA.

The imprinted Kcnq1 domain on mouse Chromosome 7 is under the control of a paternally expressed 90 kb non-coding RNA Kcnq1ot1, also known as Lit1. The syntenic human region on chromosome 11 is associated with Beckwith-Wiedemann fetal overgrowth syndrome and the cardiac disorder long QT syndrome. The majority of the genes within this 800kb domain are silenced on the paternal chromosome by Lit1. However, at least two genes escape silencing: Trpm5 (transient receptor potential protein 5) has biallelic expression and Kcnq1 (potassium voltage-gated channel, subfamily Q, member 1) has monoallelic expression in early development but switches to biallelic expression in fetal heart, liver and gut. We hypothesize that the genes that escape repression by Lit1 are under the control of regulatory elements and that the three-dimensional interactions between these elements allow the genes to be expressed on the paternal chromosome. We have confirmed that Kcnq1 undergoes a switch from mono- to biallelic expression during embryogenesis. Thus, we propose that enhancer activity is directing the biallelic expression of Kcnq1. Our bioinformatic analyses identified potential enhancer sequences. Some of these sequences have shown to exhibit occupancy of p300 during ChIP analysis. Also, these sequences showed enhancer activity in a dual luciferase assay. Further studies will be carried out in a model system in which mouse embryonic stem cells are differentiated into ventricular cardiomyocytes. This system recapitulates embryonic development and the mono- to biallelic switch. We hypothesize that Trpm5 escapes silencing in an independent chromatin domain. We identified and confirmed through ChIP analysis several binding sites for CTCF, the mammalian insulator protein, surrounding the Trpm5 promoter. The candidate CTCF binding sites have an in vivogenetic determinant.


There is a well-established epidemiological association between skeletal fragility and atherosclerosis. There is an equally recognized association between low birth weight and atherosclerosis. Through study of the recombinant congenic mouse strains Hcb-B and Hcb-C3, our laboratory has identified a peplomyotic quantitative trait locus (QTL) on mouse chromosome 4 that modulates skeletal model in response to mechanical loading. Recombination during the strains’ construction limits the QTL region to ~6.5 Mb and includes Ecf1, encoding the enzyme 1. We hypothesize that the same QTL modulates differences in response to mechanical loading in both bone and vessels and that Ecf1 is the gene underlying the QTL. Further, we hypothesize that deficient vascular modeling in Hcb-B mice leads to impaired reproductive performance. Hcb-B carotid arteries have a smaller diameter at 90 nm Hg and are less compliant to increases in luminal pressure than Hcb-C3 carotids (p=0.026, p=0.036 respectively). Hcb-B mice
also have larger fractional heart sizes (0.71 ± 0.02% vs 0.52 ± 0.03%, p<10^-3). HcB-23 heart expresses nearly 3-fold more Ece1 mRNA, p <10^-3), with the protein data showing a similar trend. NOS3 protein is also more abundantly expressed in HcB-23 hearts. HcB-8 litters are 1.8 x 5.8 x 2.0, p<10^-3) and HcB-8 pups are lighter at birth (1.2 x 2.0 g x 1.5 x 0.2 g, p<10^-20). Placeental insufficiency may account for these differences. Immunohistochemistry of day 17 placentas shows higher ECE1 and NOS3 in HcB-23. Endothelin signaling through the B type receptor is known to induce NOS3, and endothelin signaling has also been shown to promote bone growth. These findings suggest that Ece1 expression differences underlie the multi-system pleiotropy of the chromosome 4 QTL. We speculate that an impaired response to mechanical loading may be an underlying cause of preeclampsia and intrauterine growth retardation.

12 In vivo analysis of lineage segregation among enteric neural crest derivatives in the Sox10fluorescent Model of Hirschsprung disease (HSCR). Melissa A. Musser1, Jennifer C. Corpening1, E. Michelle Southard-Smith1,2. 1) Genetic Medicine, Vanderbilt University, Nashville, TN; 2) Center for Human Genetics Research, Vanderbilt University, Nashville, TN. Hirschsprung disease (HSCR) is a congenital disorder that is defined by aganglionosis of the distal gastrointestinal tract. Genetic mutations known to cause HSCR are found in the RET, EDNRB, SOX10, GDNF, and EDN3 loci. Enteric neural crest-derived progenitors migrating from the neural tube reach the foregut and then migrate in a rostral to caudal fashion to populate the entire gastrointestinal tract. HSCR phenotype is in part attributable to abnormal migration of these enteric progenitors. However, studies in mice and humans have shown that HSCR is a multigenic disorder with variable penetrance and severity. Many pediatric HSCR patients who undergo surgical resection to have the aganglionic portion of their colon removed continue to suffer from intestinal dysmotility and enterocolitis despite the presence of enteric ganglia in proximal intestinal regions. The basis of this continued dysmotility and inflammation is unknown. A recent in vitro study using Sox10fluorescent mice neural crest progenitor cells suggests that aberrant lineage segregation contributes to the HSCR phenotype (Walters et al, 2010). To determine if aberrant lineage segregation is a developmental mechanism that affects the etiology of aganglionosis in vivo, we have begun to fate map neural crest lineages in the gastrointestinal tract. Crosses between Cre transgene drivers and HSCR models, like Sox10fluorescent are being used in parallel with immunohistochemical analysis of lineage specific markers to discern how disruptions of lineage segregation contribute to the enteric nervous system abnormalities like HSCR.

13 Sleeping Beauty Transposon mutagenesis: An alternative gene discovery method for autoimmune disease. Colleen M. Elso1, Sean T. Ivory1, Michelle Ashton1, Tony Papenfuss2, Tom C. Brodnicki1. 1) St Vincent's Institute, Melbourne, Vic, Australia; 2) Walter and Eliza Hall Institute, Melbourne, Vic, Australia. The Sleeping Beauty (SB) transposon mutagenesis system provides a powerful tool for the random disruption and rapid identification of genes in the mouse. The nonobese diabetic (NOD) mouse is predisposed to different autoimmune diseases with complex genetic aetiology, including type 1 diabetes, Sjogren's syndrome, and thyroïdies. We have chosen to perform transposon mutagenesis in the NOD mouse with the aim of identifying genes important to autoimmune pathogenesis. Mobilisation of the SB transposon is mediated by SB transposase through a 'cut-and-paste' mechanism resulting in excision of the transposon and insertion elsewhere in the genome. The transposon contains a polya trap with a green fluorescent protein (GFP) reporter gene, which is activated when the transposon 'jumps' into a gene, resulting in a potentially mutagenic event. Ten GFP+ mice have been identified, confirming that the SB tagged-mutation system is functional in the NOD mouse. The insertion site in at least seven of these mice has been mapped. Mice carrying an insertion in Ser2ic, a gene important for the production of membrane lipids, have increased levels of anti-nuclear antibodies, a marker of autoimmune pathology. This mutagenesis strategy may lead to new insights into autoimmune pathogenesis, and demonstrates the utility of SB transposon-mutation for disease gene discovery, which can also be applied to other mouse models of complex genetic disease.

14 A Phylogeny Viewer for the Dynamic Visualization and Comparative Analysis of Murine Genomes. Jeremy Wang1, Hyuna Yang2, Fernando Pardo-Manuel de Villena1, Gary Churchill3,4, Leonard McMillan1. 1) University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) The Jackson Laboratory, Bar Harbor, ME. We have developed a web-based genome browser for visualizing and accessing genotype data collected for 100 classical laboratory, 62 wild-derived mouse strains, and 36 wild-caught mice to be released in an upcoming Nature Genetics paper (Yang et al. 2011). This study resolves controversies regarding the subspecific origin of the laboratory mouse and provides the first detailed view of the haplotype diversity in most extant inbred strains. We have augmented the SNPs and known genes with a mosaic of intervals representing subspecific origin, shared haplotypes, and local phylogeny. The primary goal of our tool is to allow users to visualize and interact with this data set to aid in the comparative genome analysis. Unlike existing browsers, our tool allows visualization of multiple collinear genomes to better facilitate comparative analysis. The visualization methods change dynamically based on genome resolution along with subset and order of selected strains. Over large regions, small-scale features are compressed to provide compound representations to better aid in understanding the data. Strains can be locally sorted according to subspecific origin or haplotype identity at any user-selected position. Haplotype intervals can also be dynamically re-colored to best visualize haplotype similarity. We partition the genomes into intervals which exhibit little evidence of historical recombination, enabling the analysis of global and local similarity. Analyses of these variations will be extremely valuable for improved mapping resolution and analysis of differential gene expression, disease susceptibility, and other QTL approaches. Using our local phylogeny and haplotype structure we can very accurately predict the quantitative trait state of our large set of strains given a small sample set for variations across the genome. This tool is undergoing constant development and improvement, and is a valuable resource for comparative genome analysis in the mouse.

15 Targeting Plasmacytoma Susceptibility Pathways in Mice Predicts Effective Combination Treatment for Multiple Myeloma Patients. Jonathan T. Papenfuss1, Carman L. Apel1, Can Chen1,2, Shengli Jin1,2,3,4, Li Q1,2,5, Datian Wang1,2,5, Christine H. Naylor1,2, Brian J. Holyoke1,2,3,4, B. Mock, CCR, NCI, National Institutes of Health, Bethesda, MD. Multiple myeloma (MM) and murine plasmacytoma (PCT) are rare mature B-lymphoid malignancies. Allelic variants of MTOR and p16 affect susceptibility to PCT, and abnormalities like HSCR. Multiple myeloma (MM) and bone diseases have shared haplotypes, and local phylogeny. The primary goal of our tool is to allow users to visualize and interact with this data set to aid in the comparative genome analysis. Unlike existing browsers, our tool allows visualization of multiple collinear genomes to better facilitate comparative analysis. The visualization methods change dynamically based on genome resolution along with subset and order of selected strains. Over large regions, small-scale features are compressed to provide compound representations to better aid in understanding the data. Strains can be locally sorted according to subspecific origin or haplotype identity at any user-selected position. Haplotype intervals can also be dynamically re-colored to best visualize haplotype similarity. We partition the genomes into intervals which exhibit little evidence of historical recombination, enabling the analysis of global and local similarity. Analyses of these variations will be extremely valuable for improved mapping resolution and analysis of differential gene expression, disease susceptibility, and other QTL approaches. Using our local phylogeny and haplotype structure we can very accurately predict the quantitative trait state of our large set of strains given a small sample set for variations across the genome. This tool is undergoing constant development and improvement, and is a valuable resource for comparative genome analysis in the mouse.
Sequence variation amongst 17 classical and wild-derived mouse genomes and its affect on gene regulation and phenotypic variation. David J. Adams1, Thomas Keane1, Leo Goodstadt2, Pet Danecik1, Breys payseur1, Michael White1, Kim Wong1, Binnaz Yalcin1, Andreas Heger1, Avi Agam2, Christoffer Nellaker3, Martin Goodson4, Nick Futro5, Eleazar Eskin1, Ian Jackson2, Richard Mot1, Laura Reinholdt1, Chris Ponting1, Fawn Birney1, Jonathan Flint1. 1) Wellcome Trust Sanger Institute, Hinxton, United Kingdom; 2) The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford, OX3 7BN, UK; 3) Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, USA; 4) MRC Functional Genomics Unit, Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK; 5) University of California, Los Angeles, CA 90095-1596; 6) Medical Research Council Human Genetics Unit, Crew Rd, Edinburgh, EH4 2XU, UK; 7) The Jackson Laboratory, Bar Harbor, Maine 04609 USA; 8) European Bioinformatics Institute. Wellcome Trust Genome Campus, Hinxton. Cambridge, CB10 1SD, UK. We report genome sequences of 17 strains of laboratory mice [C57BL/6NJ, C3H/HeJ, CBA/J, A/J, AKR/J, DBA/2J, LP/J, BALB/cJ, NZO/HILJ, NOD/ShiLtJ, CAST/EiJ, PWK/PhJ, WSB/ESJ, SPRET/EiJ, 129S5SvEvBrd, 129P2/OlaHsd, 129S1/SvImJ] and identify almost ten times more variants than previously known including SNPs, SVs and transposon element insertions. We use these genomes to explore the phylogenetic history of the laboratory mouse and to examine the functional consequences of allele specific variation on transcript abundance, revealing that at least 12% of transcripts show a significant tissue specific expression bias. By identifying candidate functional variants at 897 PWK/PhJ, WSB/EiJ, SPRET/EiJ, 129S5SvEvBrd, 129P2/OlaHsd, 129S1/SvImJ...
23 Evaluating the ability of paired-end RNA-Seq to detect isoform level expression differences in the striatum of the C57BL/6J and DBA/2J inbred mouse strains. Daniel Bottomly, Nikki Walter, Priscila Darakjian, Sunita Kawane, Xusheng Wang, Robert P. Searles, Robert W. Williams, Kari J. Buck, Shannon K. McWeeney, Robert Hitzemann. 1) Research Service, Veterans Affairs Medical Center, Portland, OR; 2) Oregon Health & Science University, Portland, OR; 3) University of Tennessee Health Science Center, Memphis TN.

Alternative splicing is widely recognized as a common mechanism for the diversification of the proteome. Paired-end high throughput sequencing technology produces a relatively unbiased collection of alternative splicing events. Here we evaluate the ability of paired-end RNA-Seq data in terms of counts, abundances and effect sizes, to detect differences in transcript isoforms in the striatum of the C57BL/6J (B6) and the DBA/2J (D2) mouse strains. RNA-Seq data were generated from a single Illumina flowcell. As the B6 genome is currently the only one publicly available, we utilized a dense D2 SNP map (R. Williams pers. comm.) to create an approximation of the D2 genome and used TopHat (Trapnell et al. 2009) to align the reads to the appropriate genome. We found over 200,000 junctions in the 3’ B6 and D2 samples with about half being concordant among all samples for each strain. We found a strong correlation between concordance of splice detection and read count. Examining the distribution of junction read counts indicates that we can reliably detect only the top 50% of expressed isoforms at the given sequencing depth. We used this to look at differences in isoform abundance between the two strains. In all cases, the putative isoforms detected with strain-specific expression were those with a large effect size (high counts of an isoform in one strain which had almost no counts in the other). Overall, at the current level of sequencing, the comparison of alternative splicing between samples and strains was limited to only the isoforms with relatively high expression and with large differences. Supported in part by AA 11034, AA 13484, AA 10760, MH 51372 & VA Research.

24 TNN13K Localizes to the cardiomyocyte Z disc and requires its kinase activity for its effect on cardiomyopathic progression. Hao Tang, Lan Mao, Howard Rockman, Douglas Marchuk. Duke University, Durham, NC.

TNN13K is a cardiac specific kinase whose biological function remains largely unknown. We have recently shown that TNN13K expression greatly accelerates cardiac dysfunction in mouse models of cardiomyopathy, indicating an important role in modulating disease progression. This study presents initial molecular characterization of TNN13K protein and its kinase activity. Firstly, using antisera to mouse and human TNN13K, we show that TNN13K localizes to the sarcomere Z disc, primarily inside the desmin ring surrounding the disc; our data also suggest that TNN13K may anchor to the Z disc through its interactions with cytoskeletal actin and a Z disc scaffolding protein, myotilin. Secondly, using an in vitro kinase assay and proteomics analysis, we show that TNN13K is a dual-function kinase with Tyr and Ser/Thr kinase activity. To further investigate TNN13K kinase activity in vivo, we have generated transgenic mice expressing both wild-type and kinase-dead versions of the human TNN13K protein. Importantly, we show that the increased TNN13K kinase activity in mouse cardiac hypertrophy and the kinase activity contribute to disease progression in a cardiomyopathic mouse model. We demonstrate the clinical relevance of these observations by identifying two potential missense mutations near the kinase activation loop of TNN13K in idiopathic dilated cardiomyopathy (DCM) human patients. These combined data indicate that TNN13K is a sarcomeric Z disc kinase that mediates cytoplasmic signaling to sarcomeric structural proteins to modulate cardiac response to stress. TNN13K may play an important role in cardiomyopathy, and has strong potential as a pharmacological target of kinase inhibitors for heart disease.


TGFBI plays a central role in many diseases, including cancer, cardiovascular disease, autoimmunity and fibrosis. To interrogate biological mechanisms that regulate TGFBI biology in vivo, we previously undertook modifier screens in order to genetically map and characterize variant loci that rescue C57 Tgfb1-/- embryos from prenatal lethality due to vascular dysgenesis. In generating NIH mice that are congenic for Tgfb1-/-, we found a 1.2Mb region of 129 genomic DNA at another modifier locus, Tgfb2, on mouse Chromosome 1. This 129 genomic “contaminant” at Tgfb2 was present despite multiple generations of backcrossing to NIH. We assume that this 129 variant had been co-selected with the Tgfb1 allele. Tgfb1-/- mice significantly reduced the penetrance of NIH Tgfb1-/- prenatal lethality (P= 2 x 10^-4). A SNP screen for genetic modifiers of disease severity in the human TGFBI spectrum vasculopathy, Hereditary Hemorrhagic Telangiectasia (HHT), demonstrated that one of the five genes within synteny TGFBM2 associates with disease severity (P=2 x 10^-4), as ascertained by presence versus absence of pulmonary arterio-venous malformation (PAVM) in HHT cohorts from both the Netherlands and France. Indeed, the strongest genetic association in PAVM in HHT was seen within TGFBR2, despite screening over 70 other genes encoding known TGFβ signaling pathway or response components. This gene modulates TGF-β signaling and angiogenesis in vitro. Screening for genomic regions selectively inherited on a mouse gene KO background may be a general route to identify novel genetic modifiers of signaling and developmental pathways, as well as genetic interactions that contribute to human disease susceptibility.

26 Loss of the BMP antagonist, SMOC-1, causes Ophthalmal-Acronemic (Waardenburg Anophthalmia) Syndrome in Humans and Mice. Joe Rainiger, Ellen van Beusekom, Lisa McKie, Jacqueline Ramsay, Ian J. Jackson, Han Bruneman, Dagmar Wieczorek, Hans van Bokhoven, David R. FitzPatrick. 1) Medical & Developmental Genetics, Mammalian Genetics Unit, Edinburgh, City of Edinburgh, United Kingdom; 2) Department of Human Genetics, Institute for Genetic and Metabolic Disorders and Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, The Netherlands; 3) Institut fur Humangenetik, Universitatsklinikum Essen, Hufelandstr. 55, 45122 Essen, Germany.

Ophthalmal-Acronemic syndrome (OAS), also known as Waardenburg Anophthalmia syndrome, is defined by the combination of eye malformations, most commonly coloboma, and cleft palate occur in Smoc-1 mutant, a mouse model for the human cystic kidney disorder NPHP9. This mutant develops severe CKD by P21 and also showed a 3-fold increase in renal cystic potential compared to wild-type. These combined data indicate that Smoc-1 is a novel therapeutic target for CKD, we tested the effects of small molecule Hh inhibitors in a cystogenic kidney explant assay. In the presence of cAMP, cultured aln, Gli2-/-, activator of Hh signaling, prevents cyst formation, implicating a role for increased Hh signaling in the etiology of aln cystogenesis. To explore whether the Hh pathway might present a novel therapeutic target for CKD, we tested the effects of small molecule Hh inhibitors in a cystogenic kidney explant assay. In the presence of cAMP, cultured aln kidneys exhibited a 3-fold greater cystogenic potential than wild-type. This was completely prevented in the cystogenic kidney aln inhibitors Gant61 and Sant2. Surprisingly, the small molecules also abrogated the background cAMP-induced cysts in wild-type kidneys. We thus questioned whether the preventive effects of Gant61 and Sant2 might extend to mouse kidneys. NPHP9 mutant, a mouse model for the human kidney disorder NPHP9. This mutant develops severe CKD by P21 and also showed a 3-fold higher cystogenic potential than wild-type using the explant assay. This increased cystogenesis was dramatically reduced by both Gant61 and Sant2. Our results indicate a potential therapeutic role for small molecule Hh antagonists in CKD and suggest their potential for targeted therapy. This work also demonstrates the utility of using mouse genetic analysis for understanding human disease.
Consideration of the plate positions of the samples indicated a number of off-by-one and off-by-two errors, likely the result of pipetting errors. Such sample mix-ups can be a problem in any genetic study. As we show, eQTL data allow us to identify, and even correct, such problems. This work was supported in part by NIH grants GM074244 (to KWB) and GM067244 (to KWB).

Individuals whose predicted eQTL genotypes (based on their expression data) did not match their observed genotypes, and then went on to identify other individuals whose classifier for predicting an individual's eQTL genotype from its gene expression value. By considering multiple eQTL and their related transcripts, we identified numerous classifier for predicting an individual's eQTL genotype from its gene expression value. By considering multiple eQTL and their related transcripts, we identified numerous

predict gene candidates. We have benchmarked the system against a set of genes with known phenotype and present them here.

KOMPCluster: A Pattern Recognition and 3D Visualization System for Phenotyping Projects. Eric K. Engelhard1, Kent Lloyd1, David West1,2. 1) Mouse Biology Program, University of California, Davis, CA; 2) Children's Hospital of Oakland Research Institute, Oakland, CA.

The Knockout Mouse Program (KOMP) Phenotyping Pilot is an effort to create and characterize 312 unique mutant lines from targeted embryonic stem cells developed by the CSD consortium (Children's Hospital Oakland Research Institute, Sanger Institute, and UC Davis) and Regeneron Pharmaceuticals. Inherent to the goal of this effort is the novel list of putative tumor suppressor genes independently generated from the Retrovirus Tagged Cancer Gene Database (RTCGD). The resulting analysis revealed a cluster containing three of the aforementioned tumor suppressors (Taf1, Tpmnps4, and Slc4a4) among five members (p = 0.0013). Interestingly, a fourth member of the same cluster (Hhip2) was identified as a homolog to a human tumor suppressor candidate, a gene consistently deregulated in gastric carcinomas. This gene cluster was entirely defined by the MiniPromoters tested expressed in the brain, and 50% of those showed expression related to the gene from which they were designed.

In one specific example, Set

Mouse Gentrepid, a webserver tool for candidate gene prediction in mice and humans. Sara Ballou1,2, Jason Liu1, Martin Qiu1, Bruno Gaeta2, Duncan Sparrow2, Sally Dunwoodie2, Merridee Wouters2. 1) Structural and Computational Biology, The Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 2) School of Computer Science and Engineering, University of New South Wales, Sydney, NSW, Australia; 3) Centre for Molecular and Biomolecular Informatics, Radboud University Nijmegen Medical Centre, Nijmegen,The Netherlands; 4) Developmental Biology Division, The Victor Chang Cardiac Research Institute, Sydney, NSW, Australia; 5) School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia.

Candidiate disease gene prediction is a rapidly developing area of bioinformatics research with the potential to deliver great benefits to human health. Through the use of protein pathways and domain analyses, the Gentrepid tool identifies and ranks genes which may play important roles in human disease. Cloning this system for mouse (Mus musculus) will aid in the identification of genotype/phenotype relationships of mouse ENU mutagenesis studies by predicting affected genes. Additional predictive and prioritizing power may be obtained for human disease gene discovery by integrating mouse data into the system to ameliorate the lack of human genotype/phenotype data. Here we have developed the Mouse Gentrepid tool which utilizes known phenotype-genotype data from protein interactions, protein pathways and protein domains along with phenotype ontology to predict gene candidates. We have benchmarked the system against a set of genes with known phenotype and present them here.

We present an iterative process for mutation nomination, based on linkage data, SNP quality, allele ratios, overlap with ReSeq / Ensembl exons, splice sites, and known SNPs (dbSNP) and comparison of unrelated exome re-sequencing data sets. Importantly, we have successfully used this approach to identify and validate both spontaneous and induced mouse mutations.

28 Functional genomics in mice by whole exome sequencing. Laura Reinholdt1, David Bergstrom1, Steve Murray1, Michelle Curtain1, Heather Fairfield1, Carol Bult1, Joel Richardson1, Lucy Rowe1, Mary Barter1, Daniel Gerhard1, Mark D'Ascenzo2, Todd Richmond2, Tom Albert2, Jeffrey Jeddeloh1, Mona Spector1, Sylvia He1, Scott Lowe1, Jay Shendure1, Leah Rae Donahue1. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Roche NimbleGen, Inc, Madison, WI; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; 4) University of Washington, Department of Genome Sciences, Seattle, WA USA.

Currently, the sequencing and analysis of whole mammalian genomes remains burdensome and expensive for many laboratories. Targeted re-sequencing is less expensive and the data are more manageable, but this technique requires substantial genetic mapping and the design and purchase of custom capture reagents (i.e. arrays or probe pools). Targeted re-sequencing of the coding portion of the genome, i.e. the ‘exome’, provides an opportunity to cost-effectively identify protein-altering mutations with minimal mapping data and eliminates the need for a custom array / probe pool for each mutant. This approach is particularly relevant for the rapid analysis of large sets of mutant mice. We have developed a sequence capture probe pool representing the mouse exome. This exome is a collection of 203,225 exonic regions, including miRNAs, comprising just over 54.3 Mb of target sequence. The unique design consists of non-redundant gene predictions from NCBI, Ensembl and Vega. Our data show that capture sensitivity is high and that genetic background only modestly impacts capture performance based upon the C57BL/6j (mm9) reference. We found that approximately 45 million, 76 bp paired end reads were sufficient to provide at least 5 reads coverage of 97% of target bases, which is sufficient for detection of homozygous alleles. We present an iterative process for mutation nomination, based on linkage data, SNP quality, allele ratios, overlap with ReSeq / Ensembl exons, splice sites, and known SNPs (dbSNP) and comparison of unrelated exome re-sequencing data sets.

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Mapping vQTL: detecting major genetic loci controlling phenotypic variability in experimental crosses. William Valdar1, Lars Rönnegård2. 1) Department of Genetics, UNC Chapel Hill, Chapel Hill, NC; 2) Statistics Unit, Dalarna University, SE-781 70 Borlange, Sweden.

Traditional methods for detecting genes that affect complex diseases in humans or animal models, milk production in livestock or other traits of interest, have asked whether variation in genotype produces a change in that trait's average value. But focusing on differences in the mean ignores differences in variability about that mean. The robustness, or uniformity, of an individual's character for, eg, blood pressure in animal models of heart disease, litter size in pigs, flowering time in plants, is not only of great practical importance in medical genetics and food production but also of scientific and evolutionary interest. We describe a method to detect major genes controlling the phenotypic variance, referring to these as vQTL. Our method uses a double generalized linear model with linear predictors based on probabilities of line origin. We evaluate our method on simulated F2 and Collaborative Cross data, and on a real F2 intercross, demonstrating its accuracy and robustness to the presence of ordinary mean-controlling QTL. We also illustrate the connection between vQTL and QTLs involved in epistasis, explaining how these concepts overlap. Our method can be applied to a wide range of commonly used experimental crosses and may be extended to genetic association more generally.

Genetic Effects at Pleiotropic Loci are Context-Dependent with Consequences for the Maintenance of Genetic Variation in Populations. Heather A. Lawson1, Janet E. Cady1, Char lyn Partridge1, Jason B. Wolf2, Clay F. Semenkovich1, James M. Cheverud1. 1) Anatomy and Neurobiology, Washington University School of Medicine, St Louis, MO; 2) Biology and Biochemistry, University of Bath, Bath UK.

Context-dependent genetic effects, including genotype-by-environment and genotype-by-sex interactions, are a potential mechanism by which genetic variation of complex traits is maintained in populations. Pleiotropic genetic effects are thought to reflect functional and developmental relationships among traits. We examine context-dependent genetic effects at pleiotropic loci associated with normal variation in multiple metabolic syndrome (MetS) components (obesity, dyslipidemia and diabetes-related traits). MetS prevalence is increasing in Western societies and, while environmental in origin, presents substantial variation in individual response. We identify 23 pleiotropic MetS quantitative trait loci (QTL) in an F16 advanced intercross between the LG/J and SM/J inbred mouse strains (Wustl:LG,SM-G16; n = 1002). Half of each family was fed a high-fat diet and half fed a low-fat diet and additive, dominance and parent-of-origin imprinting genotypic effects were examined in animals partitioned into sex, diet, and sex-by-diet cohorts. We examine the context-dependency of the underlying additive, dominance and imprinting genetic effects of the traits associated with these pleiotropic QTL. Further, sequence polymorphisms (SNPs) between LG/J and SM/J as well as differential expression of positional candidate genes are examined in these regions. We show that genetic associations are different in different sex, diet, and sex-by-diet settings, indicating that context-dependency is an important aspect of pleiotropic connections among complex MetS components. We show that over- or underdominance and ecological cross-over interactions for single phenotypes may not be common, however multidimensional synthetic phenotypes at loci with pleiotropic effects can produce situations that favor the maintenance of genetic variation in populations.
The AcB61 and AcB60 recombinant congenic strains of inbred mice: susceptibility and resistance to a mouse model of human typhoid fever.

Host response and novel targets for an antimalarial HDT.

Effect of sequence variation on protein function to identify candidate eQTGs within intervals. To test predictions, we developed an in vitro screening technologies and two using candidate gene approach (Ank1-1034710 and beta1-Spectrin-1045106). We are currently conducting analyses to identify the functional basis of the mutations. Depending on the mutation, reductions in parasite growth or increased red cell production are involved in the resistance phenotypes. Therefore, the various mutations are likely to target a range of mechanisms that contribute to the host response to infection. We predict this approach will provide new insights into malaria host response and novel targets for an antimalarial HDT.

The AcB61 and AcB60 recombinant congenic strains of inbred mice: susceptibility and resistance to a mouse model of human typhoid fever. Sean Beatty, Marie-France Roy, J.C. Loredo-Osti, Danielle Malo, François Leblanc, Rebecca Leblanc, Blanche Capel. 1) Department of Cell Biology, Duke University Medical Center, Durham, NC; 2) HHMI Janelia Farm, Ashburn, VA.

Survival following acute infection with Salmonella Typhimurium, a systemic infection modelling human typhoid fever, is dependent upon a coordinated and genetically complex immune response. We have screened a panel of recombinant congenic strains (RCS) derived from reciprocal double backcrosses between A/J and C57BL/6J for their susceptibility to Salmonella infection. C57BL/6J mice carry a non-functional allele at Slc11a1 (also known as Nmp31), a major determinant of the host response to Salmonella infection. Among the RCS carrying a wild-type allele at Slc11a1, we have identified four deviant strains. The strains AcB61 and AcB62 were extremely susceptible to infection and the strains AcB60 and AcB64 were more resistant than their resistant parent, A/J. Fully informative crosses using AcB61 and AcB64 mice allowed the identification of 5 loci, Ity4 (Immunity to Typhimurium locus 4) and Ity5 in AcB61 and Ity6, Ity7 and Ity8 in AcB64. Ity5 was validated in a genetic environment free of the impact of Ity4 using a cross between A/J and ICR. Ity8 on Chr15 was validated using an (AcB60 x DBA/2)F2 population. Using genome-wide transcription profiling and fine mapping, informed through the exploitation of bioinformatics resources, we have identified candidate genes underlying the Ity5 and Ity8 loci. Using both DNA and RNA sequencing, as well as analysis of gene expression (Q-PCR) we have characterized the impact of positional candidate genes for both Ity5 and Ity8 on the host-response to Salmonella infection. Through the characterization of the Ity5 and Ity8 susceptibility and resistance loci these results provide new insight into the pathogenesis of Salmonella infection and further illuminate the genetic elements of the host immune response to the mouse model of typhoid fever.


The gonad arises as a bipotential primordium, capable of differentiating as a testis or an ovary. This fate decision depends on a complex network of interacting factors that converge on a critical threshold. To define the transcription network underlying sex determination, we took a systems genetic approach using two strains, C57BL/6J (B6) and 129S1/SvImJ (129S1), that differ in their susceptibility to sex reversal. We identified significant strain differences in the transcriptome of the E11.5 XY gonad that predict the reported sensitivity of B6 to XY sex reversal. To determine how expression levels of these genes are related, we generated a large F2 panel from B6 and 129S1 intercrosses and quantified transcript abundance in E11.5 XY gonads for 56 sex-associated genes. Using a first-order conditional independence model, we estimated a coexpression network that revealed the presence of male and female sub-networks in XY gonads. These results were consistent with traditional analyses suggesting that two opposing pathways regulate the sex determination decision. Next, we used an eQTL analysis to detect multiple autosomal regions that control the expression of key regulators of sex determination. In parallel, we conducted a fine time course analysis of expression in XX and XY gonads of both inbred strains. We consolidated this information with strain sequence data and predicted the effect of sequence variation on protein function to identify candidate eQTLs within intervals. To test predictions, we developed an in vitro gonad primary cell assay and optimized virus-based shRNA delivery methods to silence candidate genes and test for an effect on expression levels of genes mapped downstream of the eQTL. In addition to establishing a more global understanding of the complex transcription architecture underlying sex determination, we discovered novel genes that likely occupy critical nodes in the network and may underlie human Disorders of Sexual Development.

Identifying suppressors of MeCP2 symptoms in the mouse: a genetic approach to therapeutic intervention. Monica J. Justice, Christie M. Buchovcey, Frank J. Probst, Hannah M. Brown, Jill Crowe, Jay Shendure. 1) Dept Molec & Human Gen, Baylor Col Med, Houston, TX; 2) Dept Genome Sciences, School of Medicine, University of Washington, Seattle, WA.

Mutations in the methyl CpG binding protein 2 (MECP2) cause Rett Syndrome (RTT), a severe neurological disease with autistic features and developmental regression. Mouse mutants that eliminate MECP2 function provide an excellent animal model. Studies of the mouse model have shown that the symptoms in male mice can be reversed by introduction of MECP2 and partially rescued by other factors, providing substantial evidence that therapeutic intervention is possible in human patients. Although these results show promise for reversing the symptoms of RTT, gene therapy approaches with MECP2 are challenging because it lies on the X-chromosome and because it is dosage sensitive. Mouse genetics is a powerful tool to identify molecules that are important for disease suppression. We have used a mutagenesis strategy to isolate five genetic modifiers that suppress or ameliorate the symptoms of MECP2 mutation in mice. Identifying genes that reverse the RTT-like phenotype in mice will allow for the development of small molecules that may treat the disease in humans. The modifiers rescue to different extents, and rescue different phenotypes, suggesting that they alter different genes. Consistent with this notion, the modifier loci map to different genomic locations. Although we do not know that each modifier will be a good target for therapeutic intervention, they may reveal important information about the development of RTT symptoms. The first modifier gene has features that suggest the gene’s function is to remodel chromatin, and although it reveals important clues as to the function of MECP2, it may prove difficult to alter therapeutically. The second modifier gene is an excellent therapeutic target; however, our preliminary data suggest that multiple factors will be required to reverse disease entirely. Identifying additional modifiers at the molecular level may allow for drug treatments that can reverse the symptoms in children who already have disease.
Mapping of loci that modify skin tumor promotion susceptibility by divergent promoting agents. Joe M. Angel, Stuart A. McClellan, Julie Hatcher, John DiGiovanni. Division of Pharmacology & Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX. 

The majority of human cancers result from exposure to environmental carcinogens and studies indicate that common allelic variants of genes can modify the response to carcinogen exposure. These are low penetrance genes with modest effects on cancer susceptibility. The mouse two-stage skin tumor model has been used to explore the genetic bases for individual variation in cancer susceptibility to carcinogen-induced epithelial cancers. We have mapped several loci that modify susceptibility to skin tumor promotion using crossed of sensitive DBA/2 with resistant C57BL/6 mice and we recently reported that the glutathione S-transferase gene, Gsta4, is one of the tumor susceptibility genes. Genetic reductions of most MCMs below a threshold level, decreasing the number of dormant origins, cause phenotypes including genomic instability, preferential female embryonic lethality, growth retardation, decreased cellular proliferation, and cancer. We speculate that the fidelity of radiation induced DSB repair associated with LOH and tumor susceptibility are complex polygenic traits.
Pharmacological correction of cerebellar dysmorphology has a surprising salutary effect on hippocampal learning and memory tasks in the Ts65Dn mouse model of Down Syndrome. Roger H. Reeves\textsuperscript{1}, Ishita Das\textsuperscript{1}. 1) Dept Physiology, P202, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Institute for Genetic Medicine, Johns Hopkins Univ Sch Med, Baltimore, MD.

The Ts65Dn mouse is trisomic for orthologs of about half of the genes on chromosome 21. A number of morphological and physiological anomalies in this mouse recapitulate those seen in people with Down syndrome. In particular, the cerebellum and dentate gyrus are hypoplastic. We traced the cerebellar hypoplasia to the day of birth and found that trisomic granule cell precursors (gcp) have an attenuated response to mitogenic effects of Sonic Hedgehog (SHH) growth factor. This deficit is evident as a reduced rate of gcp mitosis at P0, resulting in a significant reduction in gcp at P6, about 1/3 of the way through cerebellar development. Treatment of trisomic mice at P0 with a small molecule agonist of the SHH pathway (SAG) restored the number of gcp and mitotic gcp to that of euploid mice at P6 (Roper et al., 2006). We have now determined that a single treatment with SAG at birth corrects the growth of the trisomic cerebellum throughout life. Functional deficits in tasks based on working memory and the Morris Water Maze are completely restored by SAG treatment with no apparent effect on body weight.

The laboratory mouse is the premier model for studying human biology and disease. Large-scale forward and reverse mutagenesis projects are making available mutations in every mouse gene. The phenotyping of mutant genotypes promises an explosion in analysis of gene function and in discovery and validation of new models for human disease. The Mouse Genome Informatics (MGI)” (www.informatics.jax.org) catalogues the mouse genome and links mouse genotypes experimentally determined to be models of human disease. MGI catalogs all mouse mutant alleles, with key molecular attributes, captures descriptions of phenotypes, and links mouse genotypes experimentally determined to be models of human disease with corresponding disease terms in OMIM. Standardization of nomenclature and application of bio-ontologies, including the Mouse Anatomical (MA) Dictionary and Mammalian Phenotype (MP) Ontology, ensure that data are consistently annotated, making robust searches possible. Currently >550,000 alleles are cataloged in >19,000 genes. About 28,000 genes have mouse models ranging from classical preclinical models, we are engaged in a broad industry collaboration coordinated through the Health and Environmental Sciences Institute (HESI). Isoniazid, a drug used to treat tuberculosis, was selected because it is known to cause idiosyncratic DILI leading to liver failure, and because the Drug Induced Liver Injury Network (DILIN) has recently completed whole exome sequencing on 30 patients who experienced INIH DILI. Thirty-four genetically diverse mouse strains comprising the MDP were treated with isoniazid (100 mg/kg, i.v) for one week. We observed a dose-dependent increase in incidence and severity of microvesicular steatosis (42%) and in liver triglyceride levels (15%) of strains, consistent with mitochondrial toxicity that may predispose individuals to overt hepatotoxicity.

We will show data from a mutant mouse line deficient in the regulator of actin dynamics EPS8 with resistance to diet-induced obesity. Comprehensive analysis of mice deficient for Nfatc1 revealed almost no effects on the knockout on the organism. However, these mice were largely protected from oxidative stress, blood-brain-barrier leakage, and neuronal apoptosis after cerebral ischemia. Nix4 therefore represents a novel class of drug target for stroke therapy. Mice harboring a missense mutation in the phospholipase Cγ2 gene (Plcg2\textsuperscript{g163d}) mice exhibited multiple immunological and metabolic changes including an in vitro infertility phenotype. This demonstrates the value of the Alk1\textsuperscript{-/-} mouse as a model for systemic inflammatory diseases and inflammation-related metabolic changes in humans.
Effect of genetic and environmental variation on differential DNA methylation in mouse. John D. Calaway1,3,4, John P. Didion2,3,4, Ginger D. Shaw3,4, Jason S. Spence3,4, Ron Korstanje5, Gary A. Churchill5, Fernando Pardo-Manuel de Villena1,2,3,4. 1) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC; 2) Curriculum in Bioinformatics and Computational Biology, University of North Carolina, Chapel Hill, NC; 3) Department of Genetics, University of North Carolina, Chapel Hill, NC; 4) Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; 5) The Jackson Laboratory, Bar Harbor, ME.

The methylation state of homologous CpGs varies among inbred mouse strains. We have shown previously that a number of variably methylated CpGs require the presence of genetic variation in cis. We measured DNA methylation by a modified Affymetrix Mouse Diversity Array genotyping protocol that uses heterozygous SNPs in hybrid F1s as allele-specific surrogates for nearby CpG methylation. In this study, we used a similar but computationally optimized approach and expanded the scope of the experiment by introducing environmental and age-related variables. We reciprocally crossed two highly divergent inbred mouse strains (NOD/LtJ and PWK/PhJ) in order to maximize the number of SNPs that distinguish the two parental haplotypes and to track parent-of-origin DNA methylation. Two sister experiments were conducted to study the effects of two different diets on metabolism and DNA methylation. In one of the experiments, two cohorts of mice were fed either a diet depleted of key methyl-donors (choline and folic acid) or a control diet. Both cohorts were sampled at 15 weeks of age to study acute environmental effects on DNA methylation. Mice that were fed a methyl-deficient diet were then switched to the control diet for an additional 10 weeks to study the stability of environmentally induced changes in allele-specific DNA methylation. We confirm that genetic variation in cis drives allele-specific methylation states of nearby CpGs. Our analyses include a comparison of strain-specific methylation between two tissues and between three founders of the Collaborative Cross.

Novel methods of mapping QTL, QTS and QTT for complex traits in mouse. Jun Zhu1, Zhihong Zhu1, Chenhao Zhang1, Xuseng Wang2, Lu Lu2, Robert Williams2. 1) Institute of Bioinformatics, Zhejiang University, Hangzhou, Zhejiang, China; 2) Health Science Center, University of Tennessee, Memphis, US.

It is a challenge to develop efficient statistical methods for mapping genes underlying complex traits. We have developed a new mapping approach that integrates the detection of gene-to-gene interaction and gene-to-environment interaction for quantitative trait loci (QTLs) based on marker polymorphisms, for quantitative trait nucleotides (QTNs) based on SNPs, and for quantitative trait transcripts (QTTs) based on variation in expression of transcripts. This genetic model can include cofactors (i.e. sex, age), genetic main effects (additive A, dominance D), epistasis effects (additive by additive AA, additive by dominance AD, dominance by dominance DD), and gene-to-environment interaction (AE, DE, AAE, ADE, and DDE). Mixed linear model approaches are used for unbiased prediction of all these genetic main effects, epistasis effects, and gene-to-environment interaction effects. The variation contributing to these effects is estimated. Mapping software (QTLNetwork V3.0) has also been developed, which can be used under different operating systems. In order to illustrate the approaches, phenotypic and transcriptome data on anxiety assay with alcohol and stress treatments from 71 strains of the BXD family were analyzed. Using 506 markers, we detected 8 QTLs including 3 main-effect QTLs (h²=0.27) and 3 pairs of epistasis QTLs (h²=0.23). By using 2,320 SNPs, 17 QTNs were detected for 7 main-effect QTNs (h²=0.25) and 6 pairs of epistasis QTNs (h²=0.22). We used a small mapping population (188 individuals in 5 treatments), and detected only one main QTT (h²=0.07) and 2 pairs of epistasis QTTs with very small effects. The mapping results by three methods were compared for chromosome 1 and 11. Three QTTs were located within the flanking marker intervals of three QTLs detected. The QTT location are very close to one QTLs on chromosome 1. In conclusion, the approaches we developed here are capable for identifying causal variants associated with complex traits.
ENU-induced mutation in Ank1 results in hemolytic anemia and increased susceptibility to Salmonella Typhimurium infection. Kyoko E. Yuki1,5, Megan M. Eva1, Silvia Vital1,2, Marilène Paquet1, Danielle Malo1,2,3, 1) Departments of Human Genetics and; 2) Medicine; 3) Complex Traits Group of the McGill Life Sciences Complex; 4) Comparative Medicine & Animal Resources Centre, McGill University, Montréal, QC, Canada. Salmonella, a ubiquitous Gram-negative intracellular bacterium, is a food borne pathogen that infects a broad range of hosts. Salmonella Typhimurium infection in mice is a recognized experimental model resembling systemic disease and sepsis of enteric typhoid fever in humans. Our laboratory is currently using N-ethyl-N-nitrosourea (ENU) mutagenesis to identify novel genes involved in susceptibility to infection. Using a three-generation breeding scheme, we have identified a locus, Ity16 (Immunity to Typhimurium locus 16), responsible for increased susceptibility to infection on chromosome 8 initially mapping to a 20 Mb region containing 115 genes. With the use of additional mice and markers the interval was narrowed and a nonsense mutation was identified in exon 33 of the Ank1 (ankyrin1) gene. Ank1 plays an important role in the formation and stabilization of the red cell cytoskeleton. The mutation identified in Ank1 is predicted to result in a ~155 kDa truncated protein that is missing its C-terminal regulatory domain, but retains its membrane-binding and most of its spectrin-binding domain. This mutation results in severe hemolytic anemia in infected mice that is characterized by splenomegaly, hyperbilirubinemia, jaundice, and extramedullary erythropoiesis in the spleen and liver. The anemia is accompanied by decreased hepcidin expression, iron overload in liver and kidneys and lymphoid depletion in the spleen. During infection, these mice have increased bacterial load with increased levels of IL-6, IL-1 and IFNG in liver and kidneys which is associated with worsening of the anemia. The severe anemia found in these mice, along with increased bacterial loads in liver and kidneys contributes to their increased susceptibility.

Host genetic susceptibility to influenza A infections. Klaus Schughr1,1, Esther Wolk1, Tatiana Nedelko1, Heike Kollmus1, Xavier Montagutelli2, Claudia Pommereu2. 1) Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Lower Saxony, Germany; 2) Mouse Functional Genomics, Department of Developmental Biology, Institut Pasteur, Paris, France.

To identify genetic determinants of host susceptibility to infectious diseases, our laboratory is investigating the host response after infection with influenza A virus in various mouse genetic reference populations, including inbred, BXD recombinant inbred and BeG interspecific recombinant congenic strains. We identified highly susceptible strains and characterized their host defense at the molecular and cellular level. One of the highly susceptible strains, DBA/2J, succumbed to influenza virus at early times after infection. It exhibited a high viral load, elevated cytokine/chemokine levels and extended lung pathology. Using BXD recombinant inbred strains, we identified several QTLs which contribute to susceptibility and resistance. In addition, we performed a time-series of whole-genome expression studies in mouse lungs after influenza infections for up to 60 days. Our results showed that various physiological host responses, like the activation of the innate immune system, the switch from the innate to the adaptive immune response and the establishment of immunity were well reflected in the kinetics of gene expression patterns.

Collaborative Cross mice and their power to map host susceptibility to Aspergillus fumigatus infection. Caroline Durrant1, Hanna Tayem2, Binnaz Yalcin1, James Cleak1, Leo Goodstadt1, Fernando Pardo-Manuel de Villena2, Richard Mott1, Fuad A. Iraq2. 1) Wellcome Trust Centre for Human Genetics, University of Oxford, United Kingdom; 2) Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Israel; 3) Department of Genetics, University of North Carolina, Chapel Hill, NC, USA.
The Collaborative Cross (CC) is a genetic reference panel of recombinant inbred lines of mice, designed for the dissection of complex traits and gene networks. Each line is independently descended from eight genetically diverse founder strains such that the genomes of the CC lines, once fully inbred, are fine-grained homologous mosaics of the founder haplotypes. We present an analysis of the genomes of 120 lines from one cohort of the CC, between generations 6-12 of inbreeding and substantially homozygous based on high-density genotyping at 170,000 SNPs. To demonstrate the utility of the CC, we phenotyped 371 mice from 66 CC lines for susceptibility to Aspergillus fumigatus infection. The survival time after infection ranged from 4 to 28 days and varied significantly between CC lines. Quantitative Trait Locus (QTL) mapping based on survival analysis and ancestral haplotype reconstruction of the CC genomes identified genome-wide significant QTLs on Chromosomes 2, 3, 8, 10 (two QTLs), 15 and 18. Simulations show QTL mapping resolution (the median distance between the QTL peak and simulated true location) varied between 0.47 and 1.18 Mb. Most of the QTLs involved contrasts between wild-derived founder strains and therefore would not segregate in a comparison of classical inbred strains. Use of variation data from the genomes of the CC founder strains refine these QTLs further and suggest several candidate genes. These results support the use of the CC for dissecting host susceptibility to infectious disease and other complex traits.

Prdm14 alters gene expression in an expanded common lymphoid progenitor-like population prior to initiating leukemia. Stephen J. Simko1, EJ Dettman2, Bernard A. Ayanga1, Brandi L. Carlinos2, Judith F. Margolli1, Monica J. Justice1,5. 1) Pediatric Hematology/Oncology, Baylor College of Medicine, Houston, TX; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 3) Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX.

Our lab has recently shown that induced narrow overexpression of Prdm14, a pluripotency gene implicated in breast cancer chemoresistance, triggered lymphoid leukemia in 80% of mice. We hypothesized that Prdm14 would induce abnormal cellular differentiation with aberrant oncogene expression prior to the onset of leukemia. Mouse bone marrow enriched for hematopoietic stem cells was transduced with a marine stem cell virus-based vector containing Prdm14 or empty vector. Transduced marrow was transplanted into lethally irradiated mice. Prior to onset of leukemia, narrow cell suspensions underwent cell sorting for multiple hematopoietic progenitor lines, including common lymphoid progenitors (CLPs; Il7ra+ Il7r- KIT+ Sca1-). Gene expression arrays were performed on Il7ra+ Il7r- KIT+Sca1- cells from experimental and control mice. MicroRNA expression in mature tumors was also evaluated. Il7ra+ Il7r- KIT+Sca1- cells were expanded 1000-fold in mouse bone marrow transduced with Prdm14. This pre-leukemic cell population abnormally expressed known oncogenes (Mtor), pluripotency genes (Dlk1/Meg3), epithelial-to-mesenchymal transition genes, and Wnt, Ras, and PI3K/AKT signaling genes. Genes involved in B-cell maturation and transcription were downregulated. In our tumors, these expression changes were recapitulated, and additional pluripotency genes and oncogenes were overexpressed, including Prdm1, Pou5f1, and Mby. PRDM14-induced tumors also demonstrated significant downregulation of numerous microRNA tumor suppressors, including let-7e, mir-15b, mir-93, mir-101a, mir-141a, mir-148, and mir-320. These data provide the first direct evidence for the association of Prdm14 with cancer initiation in an in vivo mouse model and suggest mechanisms for Prdm14’s role in oncogenesis.

Recognition of maternal signature odor mixtures mediates the first suckling episode in mice. Darren W. Logan3,5, Lisa J. Brunel2, William R. Webb1, Tyler Cutforth1, John Ngai2, Lisa Showers1. 1) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK; 2) Department of Molecular and Cell Biology, University of California, Berkeley, CA; 3) Center for Mass Spectrometry, The Scripps Research Institute, La Jolla, CA; 4) Department of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, CA; 5) Department of Cell Biology, The Scripps Research Institute, La Jolla, CA.

Soon after birth all mammals must initiate stereotypic suckling behavior, failure to do so results in dehydrhation and death within days. The rapid and reproducible nature of suckling onset has led to its classification as an instinctive, pheromone-mediated behavior. However, the identity of the sensory cues that promote the very first suckling episode in mice is unknown, though they are thought to be maternally derived and olfactory mediated. To establish the source of these cues and their mode of detection we carried out neonatal behavioral assays in inbred strains of mice and genetic mutants deficient in olfactory signaling. To determine the mechanistic nature of the cues, we additionally performed metabolomic analysis and calcium imaging of olfactory sensory neurons. Surprisingly, we find no evidence of a classic pheromone acting to promote first suckling through a specialized neural circuit. Instead the initiation of suckling in mice is entirely dependent on variable and maleable blends of maternal “signature odor mixtures” that are recognized by plastic ensembles of canonical olfactory neurons. Unlike the hard-wired neural response to pheromones, this mechanism tolerates variability in both the signaling ligands and the detecting sensory neurons. It may maximize the probability that this first, essential behavior is successfully initiated in some mammalian parenting strategies.
Systems Genetics of Male Infertility in Extinct Collaborative Cross Lines. David L. Aylor¹, Timothy A. Bell¹, Ryan J. Buus¹, Mark E. Calaway¹, Darla R. Miller¹, Fanny Odel², Jenny Yu¹, Deborah A. O’Brien², Fernando Pardo-Manuel de Villena¹. 1) Department of Genetics, University of North Carolina, Chapel Hill, NC; 2) Department of Cell and Developmental Biology, University of North Carolina, Chapel Hill, NC.

More than two-thirds of Collaborative Cross (CC) mouse lines cease producing offspring during the course of inbreeding and are then declared extinct. This extinction rate is higher than can be explained by random causes. CC lines are descended from eight diverse inbred mouse strains, each of which produces fertile offspring, and 25 of 28 possible first-generation pairings also produce fertile offspring. Extinction peaks between five and nine generations of inbreeding. We test-crossed male mice from the terminal generation of over 100 extinct lines and found that over 40% are unable to reproduce with unrelated and fertile females, indicating male infertility as a major cause of extinction in the CC lines. We propose that male infertility and extinction in the CC is primarily due to genetic factors and specifically due to epistatic incompatibilities. There is a theoretical basis for this prediction since CC founder strains originate from three distinct \textit{Mus musculus} subspecies, and genetic incompatibilities restrict gene flow in natural populations. We used high-density genotypes to compare genome composition and allele frequencies between infertile and fertile male mice, with the goal of identifying segregation distortion that indicates potential incompatible loci. Additionally, we measured reproductive parameters in both groups to characterize mechanisms of male infertility in the CC.

Functional annotation of autism genes using genomic resources in the mouse. M. Bucan¹,², K.-J. Won¹, L. Wang², E. Clevac², B. Georgi², S. Wadhawan¹. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Penn Center for Bioinformatics, Univ Pennsylvania, Philadelphia, PA.

Autism spectrum disorders (ASDs) are a heterogeneous group of common childhood neurodevelopmental diseases characterized by anomalies in social behavior, impaired communication, as well as patterns of restricted and repetitive behaviors. Candidate gene studies, genome-wide associations and analysis of copy number variants implicated over 240 genes in the disease etiology (http://sfar.org/). Current findings support a model that common variants with a moderate effect together with rare mutations in genes involved in neuronal development underlie ASD susceptibility. To build a comprehensive catalog of rare variants associated with ASD both in the coding and regulatory regions we have sequenced 18 autistic individuals using two approaches a) Exome (38 Mb) of known exons, miRNA and ncRNA and b) Genomic regions encompassing 100 ASD-associated genes and their regulatory regions (3.5 Mb). Preliminary analysis of the sequence data has revealed ~15,000 exonic SNPs in each individual on the exome platform. We utilized data from the 1000 Genomes project to identify novel mutations in the coding and non-coding regions. Our data reveal tremendous genetic heterogeneity because each individual, including affected siblings harbors 20-70 nonsynonomous mutations in different set of known ASD-associated genes or heterozygous mutations in known Mendelian diseases. In addition to the observed coding variation we have also identified rare SNPs in mouse-human-rat conserved (potential regulatory) elements. Functional genomic resources in the mouse, including predicted p300 binding sites and CBP binding sites around neuronal activity induced genes, as well as experimentally validated neuronal enhancers, provided annotation of 500 SNPs, including 140 novel variants found in ASD children. Moreover, based on observed phenotypes of existing KO mutations in the mouse for over 70 ASD genes, we suggest novel clinical phenotypes that should be considered or evaluated in ASD children.
An ENU mouse model of a GWAS obesity gene - a neuronal-specific role in energy homeostasis. Angela W. S. Lee, Michael Schaefer, Roger D. Cox. MRC Mammalian Genetics Unit, Harwell, United Kingdom; 2) Institute of Anatomy and Cell Biology, Centre for Neurosciences, Freiburg, Germany.

Energy homeostasis is regulated by a balance between calorie intake and energy expenditure which involves the correct functioning of various metabolic tissues orchestrated by the CNS. Imbalance of this system may result in obesity. To date, genome-wide association studies (GWAS) have identified 32 novel loci for obesity and body mass-related traits. However, the molecular mechanisms of specific susceptibility genes in relation to obesity are yet to be confirmed and characterised. At Harwell, we generated an ENU mouse model for one of the GWAS genes, neuronal growth regulator 1 (NEGR1). NEGR1 is specifically expressed in brain and is known to promote neurite outgrowth in mouse brain cortex and hippocampus, suggesting the role of the CNS in regulating energy homeostasis. Our ENU mutation (I87N) lies within the C2 domain which affects membrane trafficking and the ability to form cell aggregates as shown in transfected cell assays. Mice homozygous for I87N have normal body mass up to 8wk, but at later stage display reduced body mass (+/-11%, P<0.01) due to a reduction in lean mass (+/-11%, P<0.01) and liver mass (-18%, P<0.05). Interestingly, mutants are hyperphagic as supported by an upregulation in hypothalamic orexigenic neuropeptides such as Npy. This change, however, does not correlate with a change in energy expenditure according to indirect calorimetry. Mutant liver appears resistant to developing fatty liver when fed with high fat diet. In addition, liver and muscle show an upregulation of Pck1, suggesting that glucose storage may be depleted, possibly due to preferential usage of anaerobic energy source. This is supported by glucose tolerance test which shows that mutants are more efficient in uptaking blood glucose comparing to wild-type. The current study provides evidence that confirms the link of a GWAS candidate to obesity, and demonstrates the power of large-scale GWAS approach and the use of mouse mutation to identify the underlying genes.

Estrogen receptor alpha dependent regulation of lupus susceptibility loci. Karen A. Gould, Kimberly Bynote, Jenny Fusby, Shayla Reffert. Department of Genetics, Cell Biology & Anatomy, University of Nebraska Medical Center, Omaha, NE.

Lupus is a autoimmune disease affecting ~1.5 million Americans, 90-95% of whom are women. Lupus results from a loss of immunologic tolerance and the development of autoantibodies against nuclear antigens. Although lupus susceptibility is strongly influenced by genetic factors, environmental factors also play a role in disease onset and progression. Exposure to endogenous and exogenous estrogens represents one such environmental risk factor. Epidemiological data in humans and studies using mouse models of lupus suggest that the gender bias in lupus is largely due to estrogens, which are thought to facilitate loss of tolerance and enhance antibody production. However, the molecular pathways through which estrogens exert these effects are not defined. Through analysis of estrogen receptor alpha (ERα) deficient mice on the lupus prone (NZB x NZW)F1 background, we have shown that ERα regulates the effects of estrogens on lupus pathogenesis. These data suggest that ERα modulates that action of NZB and/or NZW derived lupus susceptibility alleles. Interestingly, the impact of some susceptibility loci, such as Sle1, which controls loss of tolerance, is greater in females than males. However, the role of estrogens and ERα in mediating Sle1-dependent gender differences is not known. We hypothesize that estrogens, acting via ERα, promote lupus by modulating the action of lupus susceptibility loci such as Sle1 and directly regulating the expression lupus susceptibility genes. To test these hypotheses, we have evaluated the impact of ERα deficiency on loss of tolerance in Sle1 congenic mice. We have also evaluated the impact of ERα deficiency on the expression lupus susceptibility genes. The results of these studies indicate that ERα directly modulates the activity and expression of lupus susceptibility loci. These results imply that pharmacological manipulation of ERα using selective estrogen receptor modulators could provide a therapeutic benefit by directly targeting and downregulating lupus susceptibility loci.

Spink3 is involved in pancreatitis through the regulation of autophagy. Ken-ichi Yamamura, Kimi Araki, Masaki Ohmurraya. Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Kumamoto, Japan.

Serine protease inhibitor Kazal type 1 (SPINK1) was originally identified as a trypsin inhibitor by Kazal et al. in 1948. Linkage studies in human patients suggested the association of hereditary pancreatitis and mutation of SPINK1 gene. However, mutation of SPINK1 gene does not change inhibitor activity for trypsin. Thus, the mechanism for development of pancreatitis is unknown. To examine the role of SPINK3 (a mouse homologue of human SPINK1) in pancreatitis, we produced Spink3-deficient mice using homologous recombination in ES cells. Surprisingly, knockout mice died within two weeks after birth due to autophagic cell death in the exocrine pancreas, but not to pancreatitis. Autophagy induction was confirmed by the increased level of LC3-II in Western blot analysis and by autophagic vacuoles in electron microscopic analysis. These data indicate that the SPINK3 is a negative regulator of autophagy. To further analyze the relationship between autophagy induction and pancreatitis, we produced conditional knockout mice for the Atg5 gene which is essential for autophagy induction by mating mice with the flox allele for Atg5 and elastase I-Cre mice. As cerulein is a cholecystokinin analogue and cerulein-induced pancreatitis model is a well-established model for pancreatitis, we used this model in this experiment. We first confirmed that autophagy was not induced in pancreatic acinar cells of these mice. Interestingly, the cerulein-induced acute pancreatitis became much less severe as judged by pathologic criteria in the absence of autophagy. To analyze the role of autophagy, we analyzed trypsin activity in isolated pancreatic acinar cells. We found that trypsin activity was dramatically reduced in acinar cells from conditional knockout mice, suggesting that autophagy is involved in activation of trypsinogen. Taken together, hereditary pancreatitis in human can be explained by the mutation of SPINK1 gene may be due to excessive autophagy induction, but not to loss of trypsin inhibitor activity.

As experimental biology evolved over the past 50 years from a practice centered around use of the microscope to one dominated by molecular analysis, its practitioners designated themselves "Molecular Biologists". Such investigators established journals named after their designated molecular expertise, and universities competed for Departments of Molecular Biology. In 2011, very little retrospection is needed to recognize this fruitful marriage between the disciplines of chemistry and genetics. We can readily appreciate the enormous insights into mammalian biology that have emerged in this era, however, more thought is required to appreciate the role of the Biologist. This lecture will explore, through a prospective lens, several facets of the career of the mammalian biologist, Verne Chapman, that are important going forward. I shall discuss with you two questions: What developments in the classical disciplines of chemistry and genetics can fuel the expansion of the Molecular Genetic Era? And what marriages with new disciplines can empower the experimental mammalian biologist? For me, these questions are illuminated by three facets of Chapman’s persona: Diversity oFrom inbred strains to informative recombinant and F1 constructs oFrom the Mus and Rattus genera to human biology: Genetics and epigenetics - nature and nurture oFrom Jacob and Monod 1961 and Lyon 1961 oPolymorphisms versus mutagenesis oGenomic DNA, its modification, chromatin and cDNA oThe mammalian life cycle oFrom Mt. Desert to North America, Europe, and Asia oThe Mammalian Genome Society, the GSA and other communities: their intersections on biological planes.
POSTER: Translational biology

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

66B

Mutation of the carboxamyl-phosphate synthetase 2 (Cad) leads to embryonic lethality in mouse. Yung-Hao Ching1,2, Erin K. Stensø2, John C. Schimenti2. 1) National Laboratory Animal Center, Taipei, Taiwan; 2) Center for Vertebrate Genomics, Cornell University, College of Veterinary Medicine T9014A, Ithaca, NY 14853. Carboxamyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydorotase (CAD) is a multi-functional protein that catalyses the ATP-dependent synthesis of de novo synthesis of pyrimidine. Mutations of Cad homologs in several organisms have been reported, causing various phenotypes including impaired viability, sterility, dysmorphology and lethality. Here, we report the identification of two N-ethyl-N-nitrosourea (ENU) - induced alleles of mouse Cad (Cad<sup>α18</sup>18 and Cad<sup>α18</sup>18-18). These are the first two <i> Mus musculus</i> carrying mutant alleles of Cad to be reported. Both alleles cause peri-implantation lethality in homozygotes associated with defects in growth of the inner cell mass (ICM). Whereas cells bearing the Cad<sup>α18</sup>18-18 allele retained carboxamyl phosphate synthetase (CPase) activity, the activity was refractory to UTP inhibition. These results suggest that this mutant allele has impaired UTP feedback regulation, and that disruption of this regulation is as critical to ICM growth and embryogenesis as is CPase activity itself, which is lost in the Cad<sup>α18</sup>18-18 allele.

67C

Systems Pharmacogenomics of Antipsychotic Adverse Drug Reactions: Haloperidol and Clozapine. James J. Crowley, Corey R. Quackenbush, Darla R. Miller, Ginger D. Shaw, Yunjung Kim, Fernando Pardo-Manuel de Villena, Patrick F. Sullivan. Dept Gen, Univ North Carolina, Chapel Hill, NC. Background: All existing antipsychotic drugs come with substantial risk for severe adverse drug reactions (ADRs), causing >50% of patients to discontinue assigned treatments. Classical antipsychotics (prototype: haloperidol) cause movement disorders (tardive dyskinesia and extrapyramidal symptoms) in ~25% of patients while newer antipsychotics (prototype: clozapine) induce weight gain in ~20% of patients and a potentially fatal agranulocytosis in ~1%. The mouse represents an ideal model system to identify genetic predictors of these ADRs for subsequent testing in human subjects. Methods: In preparation for scaling up to test Collaborative Cross (CC) RIX mice, we have examined a multitude of antipsychotic ADR-related phenotypes by running a comprehensive testing battery on the 8 inbred CC parental strains and 54 F1 hybrid lines (di-allel study). For each line, and both sexes, we treated one mouse with haloperidol, one with clozapine and two with placebo (total projected N = 685, including replicates). Tests included measures of body weight, body composition, electrocardiogram, open field activity, muscualr rigidity, vacuous chewing movements, blood chemistry, hematology and pre-pulse inhibition of the acoustic startle. Results: As expected, haloperidol produced highly significant and strain specific effects on open field activity, body weight, muscualr rigidity, vacuous chewing movements and pre-pulse inhibition. Clozapine, however, failed to induce weight gain or a drop in white blood cells in any of the strains examined. Discussion: This preliminary testing battery has helped establish the most promising tests for carrying forward to CC RIX animals, wherein we expect to map QTL regulating ADR susceptibility.

68A

Molecular bases of expression of syndromic photoreceptor dystrophies by the RPGR- and cell-type-dependent subcellular sorting, tethering and processing of RPGRIP1. Paola A. Ferreira, Kyoung-in Cho, Humaid Al-Qahtani, Hao Chen, YL. Ophthalmology/Pathology, Duke Univ Med Center, Durham, NC. Human mutations affecting components of the interactome assembled by the Retinitis Pigmentosa GTase Regulator-Interacting Protein 1 (RPGRIP1) cause severe syndromic retinal dystrophies of variable penetrance and expressivity by elusive mechanisms. The Retinitis Pigmentosa GTase Regulator (RPGR), which is homologous to RCC1, a nucleotide-exchange factor for RAN GTPase interacts with the RPGRIP1 via its RHD domain, and both sexes, we treated one mouse with haloperidol, one with clozapine and two with placebo (total projected N = 685, including replicates). Tests included measures of body weight, body composition, electrocardiogram, open field activity, muscualr rigidity, vacuous chewing movements, blood chemistry, hematology and pre-pulse inhibition of the acoustic startle. Results: As expected, haloperidol produced highly significant and strain specific effects on open field activity, body weight, muscualr rigidity, vacuous chewing movements and pre-pulse inhibition. Clozapine, however, failed to induce weight gain or a drop in white blood cells in any of the strains examined. Discussion: This preliminary testing battery has helped establish the most promising tests for carrying forward to CC RIX animals, wherein we expect to map QTL regulating ADR susceptibility. Regarding clozapine, a high-fat diet may be required to detect clozapine-induced weight gain and, as seen in humans, clozapine-induced agranulocytosis may be an idiosyncratic reaction in mice.

69B

Mechanistic insights into regulation of metastasis by SIPA1. Thomas R. Geiger1, Katie Mattaini1,2, Mia Williams1,2, Renard Walker1, Jude Alsarraj1, Rosan Nieves Borges1, Kent Hunter1. 1) NCI/CCR/LCBG, National Institutes of Health, Bethesda, MD; 2) current address: Massachusetts Institute of Technology, Cambridge, MA; 3) current address: Cleveland Clinic and Case Western Reserve University, Cleveland, OH. Metastasis is the major cause of morbidity and mortality of cancer patients. Still, the molecular mechanisms underlying metastasis are incompletely understood. Conceivably, a better knowledge of the metastatic process will enable the design of better treatments for cancer patients in the future. Previously, we have shown that genetic background has a strong influence on metastasis susceptibility in mice. Quantitative Trait Loci (QTL) analysis in a mammary carcinoma mouse model identified Sia1 as a candidate metastasis gene. Our subsequent analysis demonstrated that Single Nucleotide Polymorphisms (SNPs) in the Sia1 gene are associated with metastasis in mice and human breast cancer patients. Consistent with this observation, we have shown that Sia1 regulates metastasis in breast cancer cells; however, the molecular mechanisms remain largely unknown. We identified several potential binding partners of Sia1 in a yeast-two-hybrid screen, and confirmed interactions of Sia1 with BRD4, RRP1B and the inner nuclear membrane protein SUN2 in subsequent experiments. Our analysis suggests that several complexes of Sia1 exist in different compartments of the cell. A structure-function analysis that we have begun to carry out indicates that Sia1 regulates metastasis in several ways, depending on its interaction partners and subcellular localization. Furthermore, the enzymatic function of SIPA1, a putative phosphatidylinositol 4-phosphate 5-kinase, is conserved among species, and its expression is associated with metastasis. We conclude that the triglyceride lipase ancestral gene for the vertebrate lipid family is one of three members of the triglyceride lipase family that contributes to vascular lipoprotein degradation and serves a dual role in triglyceride hydrolysis and in facilitating receptor-mediated lipoprotein uptake into the liver. Amino acid sequences, protein structures, and gene locations for vertebrate LIPC genes and proteins were sourced from previous reports and vertebrate genome databases. Mouse Lipc was distinct from other neutral lipoprotein genes (Lipc: endothelial lipase (EL) and Lpl: lipoprotein lipase (LPL)) and has been previously located on Chromosome 9 with nine coding exons. Exon 9 of human, mouse and rat Lipc genes contained 'stop' codons in different positions causing changes in C-termini length which may explain the reported lower mouse HL binding to heparin sulfate proteoglycans on liver parenchymal cells in comparison with human HL. Vertebrate HL protein subunits shared 58-97 percent sequence identities including active site, signal peptide, disulfide bond and C-acyl-lysine oxidation sites, as well as functional properties of HL (hepatic) and interferon binding regions. Predicted secondary and tertiary structures revealed similarities with the 3D structure for horse pancreatic lipase (LIP). Potential sites for regulating Lipc gene expression were observed including CpG islands near the ~5'-untranslated regions of the mouse and rat Lipc genes. Phylogenetic analyses examined the relationships and potential evolutionary origins of the vertebrate LIPC gene family with other neutral triglyceride lipase gene families (LIPC and LPL). We conclude that the triglyceride lipase ancestral gene for the vertebrate LIPC gene predicted the appearance of fish during vertebrate evolution.

70C

MOUSE HEPATIC LIPASE (Lipc): COMPARATIVE GENOMICS, PROTEOMICS AND PHYLOGENY. Roger S. Holmes1,2, Laura A. Cox1. 1) School of Biomedical and Physical Sciences, Griffith University, Brisbane, QLD, Australia; 2) Dept of Genetics, Texas Biomedical Research Institute, San Antonio, TX USA. Hepatic lipase [Lipc (mouse) or LIPC (human); H, E.C.3.1.1.3] is one of three members of the triglyceride lipase family that contributes to vascular lipoprotein degradation and serves a dual role in triglyceride hydrolysis and in facilitating receptor-mediated lipoprotein uptake into the liver. Amino acid sequences, protein structures, and gene locations for vertebrate LIPC genes and proteins were sourced from previous reports and vertebrate genome databases. Mouse Lipc was distinct from other neutral lipoprotein genes (Lipc: endothelial lipase (EL) and Lpl: lipoprotein lipase (LPL)) and has been previously located on Chromosome 9 with nine coding exons. Exon 9 of human, mouse and rat Lipc genes contained 'stop' codons in different positions causing changes in C-termini length which may explain the reported lower mouse HL binding to heparin sulfate proteoglycans on liver parenchymal cells in comparison with human HL. Vertebrate HL protein subunits shared 58-97 percent sequence identities including active site, signal peptide, disulfide bond and N-glycosylation sites, as well as potential protease ('hepatic') and heparin binding regions. Predicted secondary and tertiary structures revealed similarities with the 3D structure for horse pancreatic lipase (LIP). Potential sites for regulating Lipc gene expression were observed including CpG islands near the ~5'-untranslated regions of the mouse and rat Lipc genes. Phylogenetic analyses examined the relationships and potential evolutionary origins of the vertebrate LIPC gene family with other neutral triglyceride lipase gene families (LIPC and LPL). We conclude that the triglyceride lipase ancestral gene for the vertebrate LIPC gene predicted the appearance of fish during vertebrate evolution.
Major Histocompatibility (MHC) genes are critical mediators of the adaptive and innate immune system. These genes function in part by processing and presenting non-self and tumor antigens to the adaptive immune system. Cells presenting non-self or tumor antigens are recognized and destroyed by cytotoxic T cells in a process called immune surveillance. Many pathogens and cancer cells bypass immune surveillance by decreasing the expression of the MHC genes. Re-expression of MHC genes in cancer cells and cells infected by pathogens can re-establish immune surveillance mechanisms for therapeutic benefit. We have identified the Nucleosome Remodeling Factor (NURF) as key regulator of MHC gene expression likely through the ATP dependent repositioning of nucleosomes. We have shown that the expression of MHC genes is dependent on the NURF complex in normal and breast cancer cells. In each of the normal cell types analyzed, deregulated transcription correlates with changes in nucleosome positioning suggesting that NURF is a constitutive regulator of chromatin structure at MHC class I and II genes. Most importantly, the changes in chromatin structure occur at regulatory sequences in promoters and novel putative distal regulatory elements suggesting a causal role for NURF function in regulating MHC gene transcription. We have focused on HE-K1 where NURF functions to repress transcription likely by regulating nucleosome positioning at both the promoter and a novel upstream silencer element. Because NURF is an ATP utilizing enzyme with many active sites, these results suggest that it is a viable candidate target for the reregulation of MHC genes in cancer cells by small molecules therapeutics.

72B

Genetic background determines whether global Stat5b knockout enhances or suppresses murine hepatocarcinogenesis. Christopher Oberley, Norman Drinkwater. McArdle Laboratory for Cancer Research, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin 53706, USA.

Previous research from our lab has shown that murine hepatocarcinogenesis requires growth hormone (GH). We sought to determine if the growth hormone pulse-responsive transcription factor Stat5b is also required. We assessed the liver cancer susceptibility of global Stat5b knockout (Stat5b-/-) mice on three distinct genetic backgrounds: BALB/c, C57BL/6 (B6), and C3H/He (C3H). We complemented these tumor studies with a comparison of hepatic gene expression profiles of Stat5b-/- males to profiles of wild-type mice and resistant mutants; GH-deficient little and AR-null Tfm males. We found the effect of Stat5b knockout on hepatocarcinogenesis was dependent on the genetic background. Stat5b-null B6 congenic males and females developed approximately twice as many tumors as WT controls (p = 0.0007 and p = 0.01 respectively). Both male and female Stat5b-/- B6 congenics also developed more large tumors than WT or Stat5b+/- mice. In BALB/c congenics, however, loss of Stat5b had no effect on either sex, though Stat5b-/- males were marginally less sensitive (p = 0.03). Further, Stat5b-null C3H congenic males were resistant to liver cancer, developing roughly 2.5-fold fewer tumors (p = 0.009). Whole-genome hepatic gene expression analysis revealed similar expression profiles among Stat5b-/- males and resistant GH-deficient little and AR-null Tfm males. Half of the genes disregulated in little males were similarly altered in Stat5b-/- and Tfm males. A vast majority of the changes observed in Stat5b-/- males were also observed in Tfm males. A comparison of sensitive B6 and resistant BALB/c congenics also revealed no striking expression differences. These results demonstrate that genetic background influences whether Stat5b enhances or suppresses murine hepatocarcinogenesis.

73C

TOWARDS FINE-GRAINED PHENOTYPIC ANALYSES OF MOTOR FUNCTIONS: THE INVERSE KINEMATICS OF MOUSE GAIT PATTERNS. Satoshi Oota1, Yosuke Ikemoto2, Koh Ayusawa, Kazunori Mekada, Nobumori Kakudo, Hirokazu Imagawa, Hiroyuki Hishida, Hiromasa Suzuki, Yuichi Obata, Hideo Yokota, Ryutaro Himeura2, Yoshihiko Nakamura2, Atsushi Yoshiki1. 1) BRC, RIKEN, Japan; 2) The University of Tokyo, Japan.

Laboratory mice are ideal genetic tools for studying the central nervous system (CNS) and the peripheral nervous system (PNS) of vertebrates. While analyses on the CNS and PNS have been elaborated at the molecular level, analyses on macroscopic phenotypes are trailing far behind. As a result, serious problems hinder the translation of mouse phenotypes to human phenotypes (diseases) especially in regard to macroscopic movement disorders. Recently, the hypothesis-driven strategy has proven useful for analyzing movement disorders in laboratory mice, compensating for the traditional comprehensive battery strategy. Biomechanics is a powerful framework for acquiring detailed motor function data that the new strategy requires. Strong demand for use of the biomechanics framework to analyze motor functions in the fields of medicine, sports, and entertainment has meant that human physics-based models have been advanced over the last couple of decades. Meanwhile no fine-grained physics-based models of the laboratory mouse have been developed. Moreover, knowledge of the functional mapping of the mouse remains considerably limited. Since the human and mouse skeletal systems are evolutionarily conserved, it is theoretically possible to map in silico part of the human muscular system onto an existing mouse skeletal model according to the evolutionary relationships. We therefore developed an algorithm that homologically maps the human muscular system onto the mouse skeleton. To validate the model, we used transgenic mice (the limb tendon-specific Scleraxis(Scx)-GFP reporter line) and obtained 3D geometries of limb tendons by using 3-dimensional internal structure microscopy (3D-ISM). Finally, we performed inverse kinematic analyses on the mouse hindlimb by using conventional motion capture data.

74A

Imaging Innervation of the Urogenital Tract based on expression of a Uchl1-H2BmCherry:GFPGpi BAC transgenic. E. Michelle Southard-Smith, Nicole Fleming, Carrie B. Wiese. Medicine, Vanderbilt University Medical Center, Nashville, TN.

Ubiquitin carboxy-terminal hydrolase L1 (Uchl1) also known as PGP9.5, is expressed in migrating neural crest-derived progenitors and mature neurons in autonomic ganglia throughout the peripheral nervous system (PNS). While a broad range of neuronal transgenic reporters have been developed for analysis of the central nervous system, tools that facilitate imaging of neuronal progenitors in the PNS, particularly the urogenital system, are lacking. Expression of Uchl1 has been observed in the PNS but only a single lacZ knock-in allele that ablates gene expression has previously been available. To visualize expression of Uchl1 in living cells in the context of normal development, we generated a bacterial artificial chromosomal (BAC) transgenic that drives expression of a H2BmCherry:GFPGpi dual fluorescent reporter from the regulatory regions of this gene. The H2BmCherry moiety allows clear discrimination of individual cells and pinpoints the locations of discrete ganglia as a consequence of nuclear-localized mCherry fluorescence. Membrane expression of the GFPGpi moiety illuminates axonal processes and cell connections in autonomic ganglia. We have compared the expression of the new BAC transgenic to the knock-in allele and determined that the transgenic allele possesses regulatory elements capable of recapitulating endogenous gene expression. Our studies have traced development of innervation in the urogenital tract revealing expression of Uchl1 in bladder urothelium, pelvic ganglia, kidney pelvis, and genital tubercle. Surprisingly comparison of the Uchl1-H2BmCherry:GFPGpi BAC expression to immunohistochemical staining with the widely used PG9.5 antibody reveals that Uchl1 is restricted to a discrete subset of neuronal progenitors. This contrast to immunoreactivity detected with PG9.5 antibody and suggests that other members of the ubiquitin carboxy-terminal hydrolase gene family contribute to the broader immuno-expression pattern. Our findings demonstrate the utility of BAC transgenesis for isolating and tracing discrete neuronal populations in the urogenital system.
There is more to Solute Carrier Family 11 member 1 (SLC11A1), protein than cytokine regulation. Agnes A. Aommoyi, 1 Susan Gauthier,2 Ming-Jie Liu,1 Hee Jung Kim,2 Molly Mo,1 Lianbo Yu1, Mark D. Hewerse,2 William LaFouse,2 Philippe Gros,3 1) N Potomac, MD; 2) Department of Biochemistry, McGill University, Montreal Canada; 3) Internal Medical, Ohio State Medical Center, Ohio State University, Columbus, OH. SLC11A1, functions to flux metal cations across the phagolysosome membrane, links infections, autoimmunity and cancers. We hypothesized that transcriptional regulation of expression of SLC11A1 causes ionic perturbations to determine cell fate. Responsiveness of MOMP derived macrophages to purified TLR-2 agonist; Pam3CSK4 treatment was determined by comparing microarray gene expression profiles for Slc11a1+/- and Slc11a1+/+ BMDMs using the Illumina mouse WG 6 2.0 beadchip. Slc11a1+/- BMDMs were globally refractory to treatment and were bioactive at basal levels, by contrast, Slc11a1+/+ BMDMs were responsive to treatment and were quiescent at basal levels. In human cells, we used SLC11A1 specific RNAi to demonstrate that SLC11A1 influences IL-1 release which involves a Pyrin-Inflammasome Complex. Based on our <SLC11A1+/- microarray gene expression profiling analyses, we show that Slc11a1 regulates p62 levels at basal levels. NF-κB gene expression was higher for Slc11a1+/- BMDMs. Similarly, basal expression of M2, TAM, endoplasmic reticulum stress inducers, regulatory T cell inducers, AIM2, a DNA sensor and DNA damage response activators were higher in Slc11a1+/- macrophages. However, we propose that this IL-1B released (whether in exosome vesicles or secreted) should interact with its receptor on adjacent cells, activates the NF-κB pathway for p62 interactions with polyubiquitinated TRAF6. Interactions with speckle or aggresome dwelling p62 would facilitate cell-death or cell-survival. Now we know that all of these processes are regulated by Slc11a1 at the transcription level. Finally, basal levels of growth factor receptors were higher in Slc11a1+/- than in Slc11a1+/+ BMDMs. These data implies that Slc11a1 influences cell fate decisions and suggests a broader role for Slc11a1 than earlier reported.

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75C

Iyer, Shilpa, 1) Division of Immunology, Biostatistics and Bioinformatics, University of Chicago, Illinois, 2) Department of Pharmacology and Cell Biology, University of Chicago, Illinois. We present an analysis of the genetic architecture of 1600 heterogeneous stock (HS) rats. These animals were genotyped at 500K high quality markers and phenotyped for over 200 traits by the EURATRANS consortium, with the aim to map measures relevant to anxiety, type 2 diabetes, the cardiovascular function, vascular fragility, body morphology, basal immunology, and neuroinflammation. This HS is an outbred population descended from eight inbred strains through many generations of circular breeding, during which many recombination events accumulate. We show first that most of the phenotypes are heritable, and therefore are likely to be amenable to QTL mapping. Second, linkage disequilibrium in the population decays relatively quickly: mean r² falls below 0.1 in 4 Mb. Therefore, it is likely that on average QTLs will cover about 10-20 genes, making the rat HS a valuable resource to study the genetic basis of complex traits. However, the animals in the HS are related in a complex pedigree, which makes QTL mapping difficult because of the presence of false positive QTL s if the familial structure is ignored. We have compared two expression dependent strategies for familial structure in the HS: simple model averaging (originally developed for mouse HS, Valdar et al. 2006, Nat. Genet.) and mixed models. We implemented a version of mixed models that uses the genotypic information to estimate relatedness, and makes the most of the known ancestry of the HS by mapping inferred ancestral haplotypes. Using simulations, we show that the latter method performs better. We will present preliminary QTL mapping results for the rat HS using this method.

77B

Genetic architecture of muscle fibre properties in a cross of LG/J and SM/J strains. Andrew M. Carroll1, Abraham A. Palmer2,3, David A. Blizzard4, Arimantas Lionikas1. 1) School of Medical Sciences, University of Aberdeen, Aberdeen, Scotland, United Kingdom, AB25 2ZD; 2) Department of Human Genetics, the University of Chicago, IL 60637; 3) Department of Psychology and Behavioral Neuroscience, the University of Chicago, IL 60637; 4) The Pennsylvania State University, College of Health and Human Development, University Park, PA 16802.

Muscle fibres can be classified into distinct fibre types based on their metabolic properties and connective tissue type, which are determined by fibre-specific gene expression. Quantifying the muscle fibre composition of different strains can shed light on the genetic architecture of muscle fibre types. We compared two strains, LG/J and SM/J, which differ in muscle properties such as contractile performance, endurance and type I fibre content.

78C


Quantifying body composition in terms of lean and fat mass is an important component in modeling the regulation of body weight. When combined with models of energy balance, the relationship between lean and fat mass provides a simple means to understand body weight variation in response to macronutrient intake and energy expenditure. However, the efficacy of such models for individuals will depend on integrating the effects of genetic variation on body composition. We used a panel of Diversity Outbred (DO) mice to investigate the lean-to-fat mass relationship. Body weight and fat percentage were measured for 150 male and female mice at two time points. We found a population-averaged lean-to-fat mass ratio of 1.6. We examined changes in lean mass and fat mass over time for each individual and observed substantial individual deviation from the population-averaged results. We performed QTL mapping to identify alleles that affect body composition. Our results suggest that the population representation of body composition is significant and therefore models based on population averages may be unreliable for many individuals.

79A

GENOME ARCHITECTURE IN ISOLATED POPULATIONS OF WILD MICE. John P. Didion1, Ryan J. Buss1, Jeremy Searle2, Fernando Paro-Manuel de Villena1. 1) Department of Genetics and Lineberger Comprehensive Cancer Center, UNC Chapel Hill, Chapel Hill, NC, NC; 2) Cornell University, Ithaca, NY.

Genus Mus is an excellent model for studying mammalian speciation due to a preponderance of species, emerging new species (such as the multiple subspecies of M. musculus), and the proliferation of chromosomal races in isolated natural populations of M. m. domesticus mice. A chromosomal race is a population with a distinct karyotype, which arises due to fusion of one or more pairs ofacentric chromosomes. These fusions, known as Robertsonian (Rb) translocations, are the most common chromosomal rearrangement in the evolution of the mammalian karyotype. In humans, Rb translocations are found at high incidence, and are implicated in embryonic lethality and severe birth defects (e.g. Down syndrome, Patau syndrome). In mouse, multiple Rb translocations have become fixed to define over 100 local chromosomal races identified in the wild. These populations provide a model for studying the genetic basis of non-random chromosome segregation (centromeric drive) that is a statistical requirement for the widespread fixation of Rb translocations. We have collected hundreds of wild M. musculus samples of both standard and chromosomal races from a broad geographic range. We genotyped these mice using two different platforms to understand their genetic architecture and to investigate the genetic basis of centromeric drive. We found that the frequency of homozygosity (inbreeding) is higher in Rb races that result in standard populations, and within Rb races homozgyosity is highest at the centromeres. In diploid, diploid number is inversely correlated with the level of inbreeding. We conclude that haplotype diversity within a chromosomal race is very low and Rb races that are geographically close also have limited variation among them. We have also performed a genome scan for loci that influence the form or preferential transmission of Rb translocations and discovered multiple candidates.
used to alter the expression of its most highly connected gene, Socs2, in primary calvarial osteoblasts. The reduction in Socs2 preferentially altered the expression of PM genes (but not genes from other modules) and increased osteoblast proliferation specific genetic variants in the mouse genome that were associated with PM behavior. This analysis uncovered a single nucleotide polymorphism (SNP) on Chromosome 8 that was significantly associated with PM gene expression. This SNP was located ~20 Kbp downstream of Sfrp1, an antagonist of Wnt signaling and a known negative regulator of osteoblast proliferation and differentiation. This SNP was also found to regulate Sfrp1 expression. Causality modeling predicted that 124 of the 259 PM genes were downstream targets of Sfrp1. This work provides new insight on the genes and transcriptional networks operative in osteoblasts.

81C
Genetic determinants of a QTL linked to cardiac left ventricular mass in mouse recombinant inbred strains. Swan Gupta, MariePier Scott-Boyer, Sylvie Picard, Smita Giridhar, Christian F. Deschepper. Cardiovascular Biology, Institut de recherches cliniques de Montreal, Montreal, QC, Canada H2W1R7. Using a panel of 24 mouse AxB/BxA recombinant inbred strains (RIS), we had previously detected on chr 13 one major QTL linked to cardiac left ventricular mass (LVM). To extend this dataset, we obtained the profile of gene expression in 4 hearts of male mice from all 24 strains, using Illumina MouseRef-8 v2.0 BeadChip microarrays. Analysis of these data with R/eQTL allowed us to detect 4315 eQTLs with LOD > 3.3, 1195 of which being defined as cis-eQTLs on the basis of the probe used for gene detection being at a < 5MB distance from the eQTL peak. A subset of 34 eQTLs showing significant correlation (p < 0.05) with LVM in our RIS behaved as "quantitative trait transcripts"(QTTs), 10 of which clustered within in a 10 MB interval centered around our chr13 LVM QTL. By RT-PCR analysis of cells obtained from the A and B parental strains, we confirmed that expression of several of these genes was indeed affected in a strain-specific manner. Altogether, this suggested that a polymorphism within a common regulator could affect expression of all eQTLs in this cluster. Interestingly: 1) the combined phenotype/expression QTL on Chr13 also contains the locus of the mir-23b-27b-24-1 microRNA cluster; 2) the latter contains A vs. B polymorphisms within transcription binding sites in its promoter; and 3) at least 2 of the genes within the Chr13 eQTL cluster have a predicted target sequence for mir-27b. Experiments are under way to test whether: 1) expression of these microRNAs are dysregulated cells and organs from the parental A and B strains; and 2) these microRNAs affect the expression of genes within the eQTL cluster. Since at least 2 of the genes within the Chr13 eQTL cluster affect cardiac remodelling in genetically modified mice, their collective dysregulation may be, at least in part, responsible for differences in LVM in our RIS panel.

82A Systems-genetics analysis of parent - offspring interactions reveals maternal but not offspring genotype influence on offspring development in recombinant inbred BXD mice. Reinmar Hager, Beatrice Gini. Computational and Evolutionary Biology, Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom. In mammals, the early environment provided by mothers represents one of the most important factors determining variation in individual phenotypes and fitness. This early environment is predominantly shaped by maternal effects where the mother’s phenotype affects offspring phenotype over and above her contribution to offspring genotype through her behaviour. Both theoretical and empirical work suggests an important role for offspring and maternal genotype mutually influencing each other’s phenotype, acting either as direct or indirect genetic effects. The extent to which variation in offspring and maternal phenotype is affected by such direct and indirect genetic effects, however, has not been ascertained. Moreover, it is important to separate these effects from other sources of variation observed in offspring development and maternal phenotypes without which a clear assignment of effects of offspring versus maternal genotype is not possible. We investigated direct and indirect genetic effects on maternal behavioural traits and offspring development in recombinant inbred BXD mice using quantitative trait locus and system-genetics analyses. The existing statistical and bioinformatics tools that combine SNP, sequence and gene expression data offer an exceptional opportunity to investigate key question in Evolutionary Biology, animal behaviour and behavioural ecology but have to date not been exploited. We demonstrate that offspring development is affected by genetic variation in mothers but, surprisingly, not by offspring genotype and identify several QTL that underlie this effect. In a cross-fostering experiment, we further found evidence for genetic coadaptation between BXD mothers and their pups as predicted by theoretical work.

83B GENETIC AND STRIATAL TRANSCRIPTIONAL CHANGES ASSOCIATED WITH SHORT-TERM SELECTIVE BREEDING FOR HALOPERIDOL RESPONSE. Ovidiu D. Iancu1, Priscila Darakjai1, Barry Malmanger1, Jason Erk1, Denesa Oberbeck1, Shannon McWeeney1, Robert Hitzemann2, 3. 1) Oregon Health & Science University, Portland, OR; 2) Research Service, Veterans Affairs Medical Center, Portland, OR. Using a panel of 24 mouse AxB/BxA recombinant inbred strains (RIS), we had previously detected on chr 13 one major QTL linked to cardiac left ventricular mass (LVM). To extend this dataset, we obtained the profile of gene expression in 4 hearts of male mice from all 24 strains, using Illumina MouseRef-8 v2.0 BeadChip microarrays. Analysis of these data with R/eQTL allowed us to detect 4315 eQTLs with LOD > 3.3, 1195 of which being defined as cis-eQTLs on the basis of the probe used for gene detection being at a < 5MB distance from the eQTL peak. A subset of 34 eQTLs showing significant correlation (p < 0.05) with LVM in our RIS behaved as "quantitative trait transcripts"(QTTs), 10 of which clustered within in a 10 MB interval centered around our chr13 LVM QTL. By RT-PCR analysis of cells obtained from the A and B parental strains, we confirmed that expression of several of these genes was indeed affected in a strain-specific manner. Altogether, this suggested that a polymorphism within a common regulator could affect expression of all eQTLs in this cluster. Interestingly: 1) the combined phenotype/expression QTL on Chr13 also contains the locus of the mir-23b-27b-24-1 microRNA cluster; 2) the latter contains A vs. B polymorphisms within transcription binding sites in its promoter; and 3) at least 2 of the genes within the Chr13 eQTL cluster have a predicted target sequence for mir-27b. Experiments are under way to test whether: 1) expression of these microRNAs are dysregulated cells and organs from the parental A and B strains; and 2) these microRNAs affect the expression of genes within the eQTL cluster. Since at least 2 of the genes within the Chr13 eQTL cluster affect cardiac remodelling in genetically modified mice, their collective dysregulation may be, at least in part, responsible for differences in LVM in our RIS panel.

84C Use of Pre-Collaborative Cross Mice to Characterize the Genomics of Allergen Response. Sami N. P. Kelada1, Danielle Carpenter1, David Aylor2, Urraca Tavarez1, Kari Kubalanza1, Bailey Peck1, John Didion1, Darla Miller1, Elissa Chesler1, Gary Churchill2, Fernando Pardo Manuel de Villena2, David Schwartz2, Francis Collins1. 1) Genome Technology Branch, NHGRI, Bethesda, MD; 2) The University of North Carolina; 3) The Jackson Laboratory; 4) The University of Colorado and National Jewish Health. The Collaborative Cross (CC) is a new and powerful mouse systems genetics resource that overcomes many limitations of previous QTL mapping approaches by effectively capturing the genetic diversity of eight diverse inbred strains. We applied a house dust mite (HDM) allergen model of asthma to nascent lines of the Collaborative Cross (CC) to identify QTLs and gene expression patterns associated with allergic phenotypes. Allergic inflammation and airway hyper-responsiveness were quantified in 150 unique lines, followed by high-density genotyping using the Affymetrix Mouse Diversity array. We identified QTLs for HDM-induced airway hyper-responsiveness on Chromosome 12 and for inflammation on Chromosomes 7 and 11. Using lung tissue, we then mapped >10,000 expression QTLs (eQTLs) and identified both cis- and trans-regulators of gene expression. To connect the gene expression data with the phenotype data, we identified transcripts that are associated with each phenotype (“quantitative trait transcripts”), and then clustered the transcripts to identify gene regulatory modules. Membership in a module implies shared transcriptional control; hence were tested for enrichment of transcription factor binding sites. Novel transcription factor-target gene predictions associated with each phenotype were generated, and these provide many new hypotheses to explore in vitro and in vivo.

85A Genetic and functional analysis of infertility in high growth FVB/NJ female mice. Rashida Lathan, Thomas Adams, Juan Medrano. Animal Science, University of California, Davis, Davis, CA. The inability to reproduce is an extremely pathology that challenges survival, and is a biological process that can be investigated to discover targets for restoration of fertility and for contraception. We are studying a phenotype of infertility in a mouse model with known multigenic causes, using tools of genetics, physiology, and pathway analysis. Females are fertile when the homozygous high growth (hg) locus, a natural deletion involving Soc2, RAIDD/CRADD, and Plexin C1, is in a C57BL/6J background (C57BL/6J-hg/hg), however, complete absence of the ability to reproduce occurs when the homozygous hg locus is introgressed onto female FVB/NJ mice (FVB/NJ-hg/hg). This lead us to conclude that infertility is the result of interaction between the hg locus and the strain genome, and has provided us with a platform for testing functional pathways involved in molecular phenotype data were analyzed using an Analysis of Molecular Variance (AMOVA) approach that revealed significant genetic changes emerging between the selected lines and the founder populations. As expected, selection significantly reduced genetic diversity within both the responsive and non-responsive lines. The transcription data were interrogated using the weighted gene co-expression network analysis (WGCNA). Validation of the network topology by subsampling revealed that ~35 samples are sufficient to reliably infer the transcriptome network. We detected a group of coexpressed transcripts (module) richly annotated with neurobehavioral traits, including genes associated with neurogenesis, behavior and synaptic vesicle processes. Using a permutation procedure, we determined that genes within this module displayed significant differences in network connectivity patterns between selected lines. Examination of the Allen Brain Atlas spatial patterns of gene expression provided evidence of significant spatial co-localization for the module genes. Importantly, the same module was affected across all three genetic backgrounds, suggesting that common molecular mechanisms underlie differences in response in interest. Interestingly, changes in connectivity patterns were independent of changes in expression levels of module genes, which remained largely unaffected between the selected lines. Supported in part by HHMI 51372 and VA Research.

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that have been shown to play roles at other imprinted loci were also detected at the Meg3 DMR in e12.5 embryos and ES cells. In e12.5 embryos, modifications corresponding to active chromatin were distributed on the maternal allele, correlating to maternal-specific histone marks and proteins present at the Meg3 DMR. Discovery of these transcriptional regulators has provided new information regarding the histone marks and proteins present at the Meg3 DMR that may play a role in proper imprinting of the DMR.

87C

The degree by which Transposable Element Variants (TEVs) contribute to behavioral and physiological traits remains an open question. To address this we identified 112,000 TEVs across 17 mouse strains from over a Terabase of newly generated sequence. TEVs were inferred by two computational pipelines that considered insertions within and without the C57BL/6J lineage, detecting 44,329 and 67,734 respectively. Of the TEVs 36,999 were classified as SINEs, 40,074 LINEs and 35,062 ERVs. The ERVs were further sub-classified into the 10 most numerous families. We document the rise and fall of different TE families across mouse evolution, with ERV families having expanded at a gradually increasing rate. We show that the half-life for the conversion of full length proviral ERV elements to solo-LTRs is approximately 800,000 years, roughly one third the rate for retrotranscribing LINEs. We find that the genome of each outbred mouse can be reconstructed as a mosaic of inbred haplotypes. In the present study we have selected one of these populations (Crl:CFW(SW)-US_P08) and designed a high-throughput phenotyping pipeline to test 2000 mice for behavioural (anxiety, depression) and physiological (cardiac, respiratory) traits as well as collecting measures from numerous tissues (haematology, immunology, biochemistry). Genetic mapping is achieved by reconstructing the progenitors haplotypes from low coverage Illumina short-read sequencing data. Barcoded libraries from 96 mice are pooled and sequenced generating ~1% coverage. Known SNPs are genotyped and are used to impute the underlying haplotype structure loci at each locus. A subset of animals has also been genotyped with the Affymetrix Mouse Diversity Array (600K SNPs) to validate our genotype-by-sequence method. We will present the first results of this study.

89B
Monogenic control of gene expression in the murine hippocampus. Richard S. Nowakowski. FSU College of Medicine, Tallahassee, FL.

Gene expression data obtained from the hippocampus consortium and housed on genenetwork.org was analyzed for 99 inbred strains, including 3 distinct mouse genetic resources, the BXD and CXB recombinant inbred strains and a small mouse diversity panel (MDP). PDNN corrected gene expression data was downloaded on data was downloaded for all of the transcripts (~45,000) represented on the Affymetrix M430v2 microarray. A simple screen was designed to identify transcripts with dichotomous expression, i.e., transcripts for which >90% of the total variance could be accounted for by a single locus with a ‘high’ and a ‘low’ allele. Strains clustered according to the putative high vs the putative low allele had highly significant differences in gene expression with NO overlap in the expression levels. A total of 459 dichotomous transcripts were identified. Of these only 33 were present in all 3 groups of inbred strains (i.e., BXD, CXB and MDP), an additional 138 were present in 2 of the 3 groups, and the remaining transcripts were approximately equally distributed in one of the remaining groups. The dichotomous transcripts present in BXD or CXB had candidate eQTLs (with high significant LRS), most of which are cis-QTL, i.e., in the vicinity of the gene itself. As expected, combining SNP and sequence data from 2 or all 3 genetic resources significantly narrows the number of candidate locus. Supported by the FSU College of Medicine.
recombination varies widely among individuals and between species. Although specific sequence variants that contribute to population-level variation in recombination rate have been recently identified, the overall genetic architecture of this key trait remains poorly understood. Using a cytological assay that enables direct in situ imaging of recombination events in spermatocytes, we report a marked (~30%) difference in the genomic rate of recombination between males of inbred strains derived from two closely related subspecies of house mice (Mus musculus musculus and M. m. castaneus). To genetically dissect this difference, we generated an F2 panel of inter-subspecific hybrid males derived from an intercross between wild-derived inbred strains CAST/EiJ (M. m. castaneus) and PWD/Phj (M. m. musculus). Much of the F2 variance for recombination rate and a substantial portion of the difference between the parental strains are explained by eight moderate- to large-effect modifiers, including two transgressive loci on the X chromosome. In contrast to the remarkable disparity observed in males, female CAST/EiJ and PWD/Phj show minimal differences in recombination rate (~5%). The existence of loci on the X chromosome suggests a genetic mechanism to explain this male-biased evolution. In addition to providing key details about the genetic architecture of genome-scale recombination rate, our study lays the foundation necessary for identifying specific genes responsible for the rapid evolution of this trait.

92B
Quantitative Trait Analysis of Baseline Hematological Parameters using Developing Lines of the Collaborative Cross. Bailey Peck1, Samir Kelada1, David Aylor2, Urraca Tavarez2, Ryan Bauss1, Darla Miller1, Elissa Chesler1,3, Gary Churchill1, Fernando Pardo Manuel de Villena1, Francis Collins1. 1) National Human Genome Research Institute, NH, Bethesda, MD; 2) The University of North Carolina, Chapel Hill, NC; 3) Oak Ridge National Laboratory, Oak Ridge, TN; 4) The Jackson Laboratory, Bar Harbor, ME. Baseline hematological parameters of blood cell number and size, measured in quantitative complete blood counts (CBC), demonstrate heritability in mammals. Recent quantitative trait locus (QTL) studies in humans and mice have identified important loci for certain CBC parameters. We utilized the developing lines from the Collaborative Cross (pre-CC) to identify novel genetic loci regulating these traits. The Collaborative Cross is a new murine panel of recombinant inbred lines that harnesses the genetic diversity of eight parental strains, and yields increased power required to map various complex traits. 129 Pre-CC mice, which were derived from this cross but had not reached full homozygosity across the genome, were phenotyped using a HEMAVET Multispecies Hematology Analyzer and genotyped using an Affymetrix Mouse Diversity Array. We identified significant QTLs for mean cell volume (Chr 7 & Chr 14), white blood cells (Chr 18), monocytes (Chr 1), and percent neutrophils (Chr 11). Experimental and phylogenetic evidence suggests that the chromosome 7 mean cell volume QTL is likely driven by variation at the adult (pre-CC) to identify novel genetic loci that harness the genetic diversity of eight parental strains, and yields increased power required to map various complex traits. 129 Pre-CC mice, which were derived from this cross but had not reached full homozygosity across the genome, were phenotyped using a HEMAVET Multispecies Hematology Analyzer and genotyped using an Affymetrix Mouse Diversity Array. We identified significant QTLs for mean cell volume (Chr 7 & Chr 14), white blood cells (Chr 18), monocytes (Chr 1), and percent neutrophils (Chr 11). Experimental and phylogenetic evidence suggests that the chromosome 7 mean cell volume QTL is likely driven by variation at the adult (pre-CC) to identify novel genetic loci that harness the genetic diversity of eight parental strains, and yields increased power required to map various complex traits. 129 Pre-CC mice, which were derived from this cross but had not reached full homozygosity across the genome, were phenotyped using a HEMAVET Multispecies Hematology Analyzer and genotyped using an Affymetrix Mouse Diversity Array. We identified significant QTLs for mean cell volume (Chr 7 & Chr 14), white blood cells (Chr 18), monocytes (Chr 1), and percent neutrophils (Chr 11). Experimental and phylogenetic evidence suggests that the chromosome 7 mean cell volume QTL is likely driven by variation at the adult (pre-CC) to identify novel genetic loci that harness the genetic diversity of eight parental strains, and yields increased power required to map various complex traits. 129 Pre-CC mice, which were derived from this cross but had not reached full homozygosity across the genome, were phenotyped using a HEMAVET Multispecies Hematology Analyzer and genotyped using an Affymetrix Mouse Diversity Array. We identified significant QTLs for mean cell volume (Chr 7 & Chr 14), white blood cells (Chr 18), monocytes (Chr 1), and percent neutrophils (Chr 11). Experimental and phylogenetic evidence suggests that the chromosome 7 mean cell volume QTL is likely driven by variation at the adult (pre-CC) to identify novel genetic loci that harness the genetic diversity of eight parental strains, and yields increased power required to map various complex traits. 129 Pre-CC mice, which were derived from this cross but had not reached full homozygosity across the genome, were phenotyped using a HEMAVET Multispecies Hematology Analyzer and genotyped using an Affymetrix Mouse Diversity Array. We identified significant QTLs for mean cell volume (Chr 7 & Chr 14), white blood cells (Chr 18), monocytes (Chr 1), and percent neutrophils (Chr 11). Experimental and phylogenetic evidence suggests that the chromosome 7 mean cell volume QTL is likely driven by variation at

93C
Chromosome-wide analysis of regional and local recombination rates in six mouse species. Petko M. Petkova1, Evelyn Sargent1, Timothy Billings1, Terry Hassold2, Karl Broman3, Ken Paigen1. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Washington State University, Pullman, WA 99164, USA; 3) University of Wisconsin, Madison, WI 53706. Meiotic recombination is a fundamental biological process required for proper alignment of homologous chromosomes and their subsequent separation to form haploid gametes. Most recombination events are concentrated in highly localized genomic regions termed hotspots. The distribution of recombination events along the chromosomes is tightly regulated and is dependent on genetic background and sex. We have identified at least three levels of regulation of recombination placement - chromosome-wide, regional and local (hotspots). In this study, we mapped all recombination events on Chr 1 and Chr 11 in all possible backcrosses involving four mouse strains - C57BL/6j (B), CAST/EiJ (C), PWD/Phj (P), and WSb/EiJ (W), each having a different allele of the recombination-positioning gene Prmd9, and studied the distribution of recombination events in female and male genotypes. Meiotic lengths of chromosomes varied substantially between crosses. Regional recombination rates were similar but showed some substantial differences near centromeres and telomeres. In most crosses the females had more recombination than males except in BxP and WxP where the sex ratio was reversed. Genetic interference in general accounted for the longer female maps. However, we found that interference was practically invariable in different crosses. The reason for the sex ratio reversion was that recombination was enormously increased near telomeres in male meiosis in crosses involving PWD/Phj, providing opportunity for more double crossovers. Only a few hotspots are sufficient to account for this effect. Our further analysis showed that this effect was present in all chromosomes suggesting the role of structure features other than DNA sequence. This study shows for the first time that regional recombination rates are genetically controlled and provides a clue to further experiments mapping and identifying the underlying factors.

94A
Integrating cross-species genomic data for prioritization of candidate genes using the Ontological Discovery Environment. Vivek Philip1,2, Jeremy Jay3, Michael Langston1, Erich Baker1, Elissa Chesler1,2. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Genome Sci. and Tech. Program, The Univ. of Tenn., Knoxville, TN; 3) Dept. of Elec. Eng. and Comp. Sci., The Univ. of Tenn., Knoxville, TN; 4) Sch. of Eng. and Comp. Sci., Baylor Univ., Waco, TX.

To date there are over 4,300 published mouse Quantitative Trait Loci (QTL), of which less than one percent have been cloned. Depending upon the crosses and sample sizes used, the confidence interval (CI) around a QTL can be large, harboring numerous candidate genes. The challenge lies in the intensive process of narrowing QTL intervals and identifying causative polymorphisms. Integrating genomic data such as microarrays and cross-species comparison of genome wide association studies has proven to be a more rapid and efficient approach for the identification of candidate genes. However, such integration requires extensive data manipulation and is often only performed over a limited set of genominc inputs. Using the Ontological Discovery Environment (ODE,www.ontologicaldiscovery.org), we attempt to provide a rapid, scalable and efficient approach for the identification of candidate genes using diverse genomic data sources and multiple species. Methods: Candidate genes will be obtained from QTL analysis. The resultant set of candidate genes residing within the 1.5 LOD-drop CI of the QTL is identified with previously published QTL, GWAS and microarrays obtained from the following species: Homo sapiens, Mus musculus, Drosophila melanogaster, Danio rerio and Macaca mulatta. Using tools available within ODE, such as Jaccard Similarity, AIBBA, Phenome Map and GeneSet Graph, we perform a cross-species, cross-platform combinatorial analysis for the identification and prioritization of candidate genes. Conclusion: ODE enables a phenotype-centered gene set analysis through the combinatorial intersection of diverse genomic gene sets encompassing multiple species and platforms making it a valuable tool for the identification and prioritization of candidate genes. Supported by RO1 AA18776.

95B
Genetic Analysis of Blood Alcohol Clearance Rates in the Emerging Collaborative Cross. Christine L. Powell1, David L. Aylor2, Wendy Foulds Mathes2, Jill Steigerwalt1, Daniel Pomp2, Gary A. Churchill1, Fernando Pardo-Manuel de Villena2, David W. Threadgill2. 1) Department of Genetics, North Carolina State University, Raleigh, NC; 2) Department of Genetics, University of North Carolina, Chapel Hill, NC.

Heritable factors underlying alcohol metabolism are of considerable interest as they most likely contribute to inter-individual variations in its toxicity, sensitivity and preference. We have analyzed pre-Collaborative Cross (preCC) mice, a large panel of recombinant inbred mouse lines designed specifically for complex trait analysis, to characterize the phenotypic diversity of blood alcohol clearance rates (BAC) and to perform genome-wide association mapping to identify causative loci that influence alcohol metabolism. Male preCC mice and founder strains were administered a 3 g/kg dose of ethanol via intraperitoneal injection and tail vein blood draws were taken 30 min post-dosing followed by every hour for the next 3 hrs. Blood alcohol concentrations were measured in serum from which BAC was determined. Phenotypic distribution for BAC in preCC mice (0-677 mg/kg/hr; n = 192) provided a continuous trait distribution. Transgressive segregation was evidenced by the presence of preCC mice at the extreme ends of the distribution. Genome-wide association mapping identified a suggestive quantitative trait locus on Chr 4 (LOD 6.49; p < 0.2) that spans 126-131 Mb and harbors numerous genes. A potential candidate gene, the opioid receptor delta 1 (Oprd1), was singled-out for further study since it has been identified in human linkage studies for alcoholism and Oprd1 -/- mice have been reported to have an increase in alcohol self-administration. On a C57BL/6J background, Oprd1 -/- and control mice administered a 3g/kg dose of alcohol had significantly different BAC rates, 115 and 63 mg/kg/hr (p = 0.0004), respectively. These data support further mechanistic analysis of Oprd1 as a potential functional candidate underlying this putative QTL.
96C Chromosome Y programs cardiac androgen responsiveness and androgen receptor occupancy on genomic DNA. Samantha Praktiknio, Bastien Llamas, Marie-Pier Scott-Boyer, Sylvie Picard, Christian F. Deschepper. Cardiovascular Biology Research Unit, Institut de recherches cliniques de Montreal, Montreal (QC) Canada H2W1R7. Although most known functions of Chromosome Y (Chr Y) genes relate to either male sex determination/differentiation and/or reproductive functions, evidence indicates that they may modulate other more general functions as well. By comparing male C57BL/6J mice to the Chr Y-substitution C57.T1Y strain (whose Chr Y originates from the A/J mouse strain), we currently had reported that Chr Y polymorphisms modulate the biologic effects of perinatal testosterone in male mice. In the current study, we found that exposure to perinatal androgens was stronger in C57BL/6J than in C57.Y1 male fetuses, as evidenced by the greater anogenital distance in C57BL/6J pups. This was not the result of increased production of androgens, since neither the total content of androgens nor the abundance of mRNA transcripts of several steroidogenic enzymes were higher in testes from C57BL/6J fetuses than in C57.T1Y. Nonetheless, the differences in perinatal androgen exposure had important consequences, since endocrine manipulations blocking the effects of perinatal androgens in C57BL/6J fetuses affected how cardiac cells and genes respond to androgens later during adult life (the phenotypic responses in C57BL/6J cells and genes becoming similar to that of their C57.Y1 counterparts). To test whether Chr Y polymorphisms could alter the effects of perinatal androgens by affecting chromatin around androgen receptor binding sites, we immunoprecipitated chromatin from neonatal C57BL/6J and C57.Y1 hearts with anti-AR antibodies. Of 90 loci showing significant enrichment for AR, only 15/90 were common in both strains, whereas 25/90 and 23/90 showed enrichment only in C57BL/6J and C57.Y1, respectively. Altogether, our findings indicate that Chr Y affects some cardiac phenotypes in adult mice by altering the programming effects of androgens around birth time, possibly via epigenetic modulation of androgen receptor signaling.

97A Diversity Outbred mice: Toward an improved understanding of population structure on murine studies of pain sensitivity. Jill M. Rech1, Vivek M. Philip2, Raymond F. Robledo1, Carol J. Bul1, Elissa J. Chesler1. 1) The Jackson Laboratory, Bar Harbor, Maine 04609 USA; 2) GSBS, Functional Genomics, University of Maine, Orono, Maine 04469 USA. Pain sensitivity varies widely between subjects, and the significant influence of genetic factors on this variability is now widely appreciated. The use of inbred laboratory mice in pain genetics studies (i.e., F2 crosses, inbred strain panels, and recombinant inbred [RI] panels) has facilitated the identification of ~330 pain-related loci in the laboratory mouse to date. However, many current murine resources lack recombinatorial precision and genetic complexity, and many important contributors to human pain sensitivity may be missed. The genetic composition of the new Collaborative Cross (CC) and Diversity Outbred stock (DO) mouse populations offers increased heterozygosity and allelic diversity. We aim to characterize the utility of these new reference populations in murine studies of pain sensitivity, as related to allelic diversity and heterozygosity. Populations of BXD RL, CC, and DO mice were typed using the hot plate assay of acute thermal nociception. Coefficient of variation and width of distribution were calculated for each population’s observed phenotypic responses. These measures were compared to those calculated from publicly available experimental crosses and inbred strain panels. CC and DO populations exhibited increases in phenotypic range of thermal pain sensitivity compared to BXD and inbred strain panels. Though pronounced, the increase was less observable in DO mice, suggesting a stabilizing effect of heterozygosity, combinatorial diversity, or allelic restriction in the outbred population. Inbred, CC, and DO mice are complimentary genetic analysis tools to classical inbred strain panels. Depending on the biological question, it may be advantageous to use a combination of genetic resources for dissecting complex traits. In contrast, the outbred DO mice may more accurately represent genetic contributions to pain sensitivity generalized to heterozygous effects in humans. Supported by The Jackson Laboratory start up funds to EJC.

98B A comparison of mammalian recombination hotspots in four mouse strains at the distal end of chromosome 1. Lorin M. Roiphe, Petko M. Petkov, Kenneth Paigen. The Jackson Laboratory, Bar Harbor, ME. Meiotic recombination is required for accurate segregation of chromosomes during meiosis, produces genetic diversity, and is important in mapping new genes. Recombination is concentrated in 1-2 kb regions called hotspots that are surrounded by large regions lacking recombination. While many of the proteins involved in the molecular events of recombination are known, with the exception of the recent discovery of PRDM9, little is known about how the location or the intensity of hotspots is determined. We have mapped the recombination hotspots present in the terminal 25 Mb of mouse Chromosome 1 in the six possible F1 hybrids of the mouse strains C57BL/6J (B), CAST/EiJ (C), PWD/PhJ (P) and WSB/EiJ (W). Many of the hotspots are strain-specific as they are present in all F1 animals that share a common parental strain and absent in all other F1s. Our finding of strain-specific hotspots suggests that each mouse strain may contain its own factor or combination of factors determining hotspot specificity. This is consistent with the action of Prdm9, as each of the four strains has its own Prdm9 allele with different DNA-binding specificity. We have also found hotspots that are only active in one of the six crosses and can be considered cross-specific. In addition, some hotspots show statistically significant differences in recombination activity between crosses. The presence of cross-specific and quantitatively regulated hotspots indicates that PRDM9 is not the only regulator of hotspot activity and that additional factors determine the location and intensity of recombination in a hotspot specific manner. These could include variation in the DNA sequence of the hotspot itself, changing the strength of protein-DNA binding, or alternatively, the interaction of two or more protein factors whose concerted action is required for hotspot recognition and activity. Our investigation opens the opportunity of mapping and identifying these factors.

99C Identification of positional candidate genes for susceptibility to seizure-induced cell death on mouse chromosome 15. P. Elyse Schauwecker. Cell and Neurobiology, USC Keck School of Medicine, Los Angeles, CA. Inbred strains of mice differ in their susceptibility to excitotoxin-induced cell death, but the role of genetics in the determination of seizure-induced excitotoxic cell death remains unclear. To quantity traits that correlate with susceptibility to seizure-induced cell death in mice, we performed 20 backcrosses on C57BL/6J (B6,resistant strain) X FVB/NJ (FVB,susceptible strain) (Schauwecker et al., 2004). We previously constructed a congenic mouse, FVB.B6-Sicld2 with 71.15 Mb of B6 donor DNA on proximal Chromosome 15 in a FVB background that captured this QTL for resistance to seizure-induced cell death. Mice homozygous for B6 alleles at the donor region showed reduced susceptibility to seizure-induced cell death as compared to FVB wildtype littermates (Schauwecker, 2010). In this study, we constructed four overlapping subcongenic with smaller B6 donor regions that encompass Sicld2 on Chromosome 15 to fine map the underlying gene(s). Resultant progeny were treated with kainate and examined for the extent of seizure-induced cell death in order to deduce the Sicld2 genotypes of the recombinants through linkage analysis. While all four subcongenic lines showed reduced susceptibility to seizure-induced cell death, a nearly 75% reduction in the extent of seizure-induced cell death was observed in FVB.B6-Sicld2. To help identify candidate genes, expression profiles of hippocampi from FVB (susceptible) and FVB.B6-Sicld2 (less susceptible) mice were interrogated using the Affymetrix Mouse Exon 1.0 ST arrays. Genes that differed by twofold, with a false detection rate of ~2% and mapped to the reduced region were the primary focus of our analysis. Of over 1.2 million probe sets analyzed on the chip, only 5 of the 83 genes most differentially expressed between FVB and FVB.B6-Sicld2 were located within the boundaries of the FVB.B6-Sicld2 subcongenic interval on Chromosome 15. Ongoing efforts in confirming any gene expression changes by quantitative real-time PCR should accelerate our identification of putative positional candidate genes.

100A A flexible estimating equations approach for mapping function-valued traits. Saunak Sen1, Hao Xiong1, Evan Goulding2, Elaine Carlson1, Laurence Tecott1, Charles McCulloch1. 1) Univ California, San Francisco, San Francisco, CA; 2) Northwestern University. In genetic studies, many interesting traits, including growth curves and skeletal shape, have temporal or spatial structure. They are better treated as curves or function-valued traits. Current methods for mapping function-valued traits are mostly likelihood- based, requiring specification of the error structure. However, such specification is difficult or impractical in many scenarios. We propose a general functional regression approach based on estimating equations that is robust to misspecification of the covariance structure. Estimation is based on a two-step least squares algorithm, which is fast and applicable even when the number of time points exceeds the number of samples. It is also difficult to use the general linear functional model, changing the number of covariates does not necessitate a new set of formulas and programs. In addition, many meaningful extensions are straightforward. For example, we can accommodate missing genotype data using multiple imputation, and the algorithm can be trivially parallelized. Simulation studies indicate that the proposed method maintains the target false positive rate under the null hypothesis regardless of covariance structure. However, likelihood-based procedures with a misspecified covariance structure do not necessarily have the desired false positive rate, and may have lower power compared to our method. We illustrate our method and its advantages using circadian mouse activity data.
POSTER: Systems genetics

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

101B
Production and Use of Mice for the Center for Integrated Systems Genomics (CISGen). Ginger D. Shaw1, Jason S. Spence1, Timothy A. Bell1, Alexander Vu2, Sandra Hall2, Leonard McMillian2, Darla R. Miller1, Fernando Pardo-Manuel de Villena1. 1) Lineberger Comprehensive Cancer Center, Dept. of Genetics, UNC at Chapel Hill, Chapel Hill, NC; 2) Dept. of Computer Science, UNC at Chapel Hill, Chapel Hill, NC.
The Center for Integrated Systems Genomics (CISGen) is an NIH funded Center of Excellence in Genome Sciences. CISGen uses Collaborative Cross (CC) mice to develop new approaches for identifying genetic and environmental factors that underlie some psychiatric disorders. Here we focus on the breeding of animals for the high-throughput behavioral and phenotypic screens, as well as databases used to manage animal husbandry and phenotyping data collected from the various assays. As the CC becomes inbred, the CC lines will be crossed to make RIX lines (Recombinant intercrosses), and these mice will be sent through the testing pipeline. One aim is to develop an efficient pipeline for delivery of thousands of RIX mice that minimizes the confounding effects of litter size, pedigree relationship, etc. We estimate to test as many as 350 RIX lines (11,200 mice). The first phase of this project used the eight founder strains of the CC and the 54 viable F1 hybrids that can be generated by crossing these strains to refine the tests, generate data for analysis and to develop data tools. CISGen began in October of 2009 and to date we have completed the 8 founders and 49 F1 lines. The sociability and anxiety behavioral screen tests for the effects of three housing conditions: single housed, group housed (four mice in a cage), and enriched housed (four mice with tubes, hats, balls, and chew toys in a cage). 495 mice have been tested for sociability and anxiety and are being used for 14 strains at the three housing conditions. 220 mice from 51 strains have been tested in the pharmacogenomics branch which includes side effects of haloperidol. The CISGen breeding pipeline is also being used to generate mice for infectious susceptibility: SARS/Influenza (Ibaric, Heise), craniofacial morphology (Facebase Consortium), cancer and drug toxicity susceptibility (Threadgill).

102C
High Resolution Genome Architecture of Genetic Reference Populations: Chromosome Substitution Panels and Recombinant Inbred Strains. Petr Sinemuk1, Irin Forej1, Robert W. Williams2, Lu Lu1, Thomas E. Johnson1, Beth Bennett1, Brad Rikke1, Christian F. Descheppe2, Marie-Pier Scott-Boyer2, Gary Churchill3, Fernando Pardo-Manuel de Villena4, 1) Institute of Molecular Genetics of the ASCR, Videnska 1083, Prague, Czech Rep; 2) University of Tennessee Health Science Center, 855 Monroe Ave, Memphis, TN 38163, USA; 3) University of Colorado at Boulder, 1480 30th St, CO 80309, USA; 4) Institut de Recherches Cliniques, 110 Des Pins O, Montreal, QC H2W 2T2, Canada; 5) The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA; 6) University of North Carolina at Chapel Hill, 103 Mason Farm Road, Chapel Hill, NC 27599, USA.
We used the Mouse Diversity Array (MDA) to genotype five unique mouse resources, two panels of chromosome substitution strains (C57BL/6J-ChrP/WD/PbForel and C57BL/6/J-Nrj) and three panels of recombinant inbred strains (BxD, LxS and AxB/BxA). Each of these resources exploits the genetic diversity present between two widely used laboratory strains. Genotyping was conducted using the MDA, a platform that can simultaneously assay over 600,000 SNPs and 900,000 invariant genomic probes in a single mouse sample. Given that MDA can achieve an exceptional resolution of 4.3kb, this work provides an exceptionally high-resolution view of the mosaic architecture of these genetic reference populations. Although each of the strains was presumed to be inbred we found that residual heterozygosity is common in at least four of these populations. Because only a single male mouse per strain was genotyped it is likely that the residual heterozygosity is underestimated. Heterozygosity was variable in length and genomic location. Because some of these regions could be tagged by selection or by de novo chromosome rearrangement we determined their allele frequencies and segregation ratio in existing colonies. Finally, we also found de novo deletions, both homozygous and heterozygous, with a length ranging from 24kb to 9Mb. Many of them result in loss of exons of the known genes. The work has profound implications for all mouse reference populations.

103A
Using the JAX Diversity Outbred population to map quantitative traits: a pilot study with early outbred generations. Karen L. Svenson, Daniel M. Gatti, Gary A. Churchill. The Jackson Laboratory, Bar Harbor, ME.
We have created a new heterogeneous mouse resource in the JAX Diversity Outbred population (J:DO). This new resource provides high resolution genetic mapping which will facilitate the identification of genes underlying human disease. The J:DO was derived from partially inbred lines of the Collaborative Cross (CC). This affords the unique opportunity to replicate and validate significant disease related haplotypes in CC strains or in their F1 progeny. Taken together these two resources provide the power and resolution of an essentially infinite outbred population with the many advantages of reproducible inbred strains. The purpose of this study was to evaluate recombination in the J:DO to date and to directly test its utility for mapping quantitative traits. In our pilot study of 150 J:DO animals from generations 4 and 5, we observed an average of 244 recombination events per strain and each with an average spacing of 10.2 megabases. Animals were phenotype for a battery of metabolic traits and genotyped at 7,854 SNP loci. We have identified significant quantitative metabolic trait loci, representing control of plasma insulin, triglycerides and cholesterol, nutrient partitioning and energy balance, bone density, and liver homeostasis. The diversity of metabolic phenotypes among this relatively small number of J:DO animals is remarkable and often more extreme than observed across the 8 founder strains of the CC. Continued outbreeding of the J:DO will improve mapping resolution with minimal loss of alleles.

104B
Cocaine-induced locomotor activation and pharmacokinetics in 45 inbred mouse strains. Lisa M. Tarantino1, Robin B. Ervin1, Hongze Duan2, Steve Cook3, William C. Zamboni1, Wonil Chung1, Fei Zou1, Tim Willshire1, 1) Department of Psychiatry, University of North Carolina, Chapel Hill, NC; 2) Institute for Pharmacogenomics and Individualized Therapy, Department of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC; 3) Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC.
Inbred mouse strains are genetic reference populations that represent a rich store of phenotypic and genetic diversity. Inbred strain surveys have historically been used to determine classes of variants. We generated ~55X coverage for the DBA/2J genome using Illumina GAII and HiSeq2000 systems and ~45X using ABI SOLiD. We used six paired end classes of variants. We identified 5,600 and 8,800 structural deletions using Illumina and SOLiD or which only 1,223 were common. An analysis of Illumina subsamples at 10, 20, 30, 40, and 55X shows 0.56 M and 0.22 M indels using Illumina and SOLiD, respectively. Only 0.11 M were common. Finally, we identified 5,600 and 8,800 structural deletions using Illumina and SOLiD or which only 1,223 were common. An analysis of Illumina subsamples at 10, 20, 30, 40, and 55X demonstrates that platforms can rapidly reach a premature SNP detection asymptote that cannot be overcome simply by higher coverage. Indel and CNV detection is more challenging and even at 100-120X we appear to be far from a “full disclosure” on sequence variants even in these two strains. As is true for SNPs, each platform detects unique and genuine subsets of indels and CNVs. This incompleteness problem is compounded by the fact that assembly is biased by using a C57BL/6J scaffold and limitations of alignment approaches used to detect variants. In conclusion, sequencing data from multiple platforms with high coverage will be necessary for the next few years to extract the majority of variants, particularly large structural variants.

105C
Comparison of variant detections using whole genome sequencing of the DBA/2J mouse strain. Xusheng Wang1, Megan Mulligan1, Khyobeni Mozhui1, Lu Lu1, Zugen Chen2, Xuebing Yu1, Chong Chen1, Stanley Nelson2, William Taylor1, Robert Williams1. 1) Anatomy and Neurobiology, UTHSC, Memphis, TN 38163, USA; 2) University of Colorado at Boulder, 1480 30th St., CO 80309, USA; 4) Institut de Recherches Cliniques, 110 Des Pins O, Montreal, QC H2W 2T2, Canada; 5) The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA; 6) University of North Carolina at Chapel Hill, 103 Mason Farm Road, Chapel Hill, NC 27599, USA.
Next generation sequencing is now widely used to detect sequence and structural variants. Here we ask how coverage depth and platform affect the rate of detection of major classes of variants. We generated --55X coverage for the DBA/2J genome using Illumina GAII and HiSeq2000 systems and —45X using ABI SOLiD. We used six paired end libraries with insert lengths from 200 to 4,000 bp, and aligned against the C57BL/6J genome. We detected 4.16 million SNPs using Illumina and 4.09 M using SOLiD, of which 3.38 M SNPs were common. Unshared SNPs were validated 93% of the time by resequencing with a total yield (~97.9% true) of 4.87 M between strains. Each platform detects an large cohort of unique SNPs (15% per platform). We detected 0.56 M and 0.22 M indels using Illumina and SOLiD, respectively. Only 0.11 M were common. Finally, we identified 5,600 and 8,800 structural deletions using Illumina and SOLiD or which only 1,223 were common. An analysis of Illumina subsamples at 10, 20, 30, 40, and 50X demonstrates that platforms can rapidly reach a premature SNP detection asymptote that cannot be overcome simply by higher coverage. Indel and CNV detection is more challenging and even at 100-120X we appear to be far from a “full disclosure” on sequence variants even in these two strains. As is true for SNPs, each platform detects unique and genuine subsets of indels and CNVs. This incompleteness problem is compounded by the fact that assembly is biased by using a C57BL/6J scaffold and limitations of alignment approaches used to detect variants. In conclusion, sequencing data from multiple platforms with high coverage will be necessary for the next few years to extract the majority of variants, particularly large structural variants.

Combinatorial genetic interaction screens have uncovered detailed networks of functional relationships in lower organisms such as *S. cerevisiae* and *C. elegans*. To explore the genetic basis of self-renewal in mouse embryonic stem cells (ESCs), we constructed a combinatorial RNAi library comprised of over 16,000 pairs of short hairpin RNAs (shRNAs). The library was generated by “all-against-all” combinatorial expansion of shRNAs targeted against factors that co-precipitate with an important regulator of ESC self-renewal, the ARID1A-BAF chromatin remodeling complex. We employed a novel massively parallel sequencing strategy to identify significant genetic interactions following selection in ESCs for phenotypes associated with self-renewal. Hierarchical clustering of predicted genetic interactions revealed functional association between components of distinct protein complexes. Our combinatorial RNAi system also facilitates the de novo assembly of mammalian genetic interaction networks, and the functional identification of genetic hubs. We observe co-regulated transcriptional programs of differentiation between genetically interacting genes, as well as physical interaction at the protein level. Further advances in combinatorial library construction and sequencing technology should permit the functional mapping of even larger, higher-resolution mammalian genetic interaction networks.


Using a population of outbred heterogeneous stock mice we have identified 164 quantitative trait loci (QTL) affecting 53 diverse traits that show a parent of origin effect (PoE). We also identified expression QTLs in the hippocampus, liver and lung that show PoE. In some cases PoE eQTL map to the same loci as phenotypic PoE QTL, suggesting candidate genes. We have confirmed three genes predicted to have PoE on weight (*Manla2*), blood (*Clic5*) and the immune system (*H2-Ab1*) for which knockout mice are available by performing reciprocal F1 crosses. We have measured gene expression using RNAseq in the crosses to determine PoE gene expression differences due to POE specific expression of the knockouts. Using this strategy we can identify causal genes under PoE QTLs and begin to dissect the responsible pathways.

Hermansky-Pudlak syndrome (HPS) is a rare recessive disorder caused by defects in the biogenesis of lysosome-related organelles. The classical triad of HPS defects includes oculocutaneous albinism, prolonged bleeding, and ceroid pigment accumulation in lysosomes. Mice homozygous for the cno allele, a frameshift mutation in the cappuccino gene, display a severe HPS-like phenotype. Our previous studies suggested that this gene is expressed in bone. We also previously observed a decrease in areal bone mineral density in cno null mice as compared to controls. To better understand this phenotype, we examined in vitro mineralization by osteoblasts from both cno+/cno and cno/cno mice. In short, calvarial osteoblasts were isolated from neonatal cno/cno and cno+/cno mice and cells were stained for alkaline phosphatase at day 14 of culture, and for mineral at day 20. While there was no difference between the two genotypes with regards to the number of alkaline phosphatase positive colonies observed, there was an increase in the number of mineralized nodules produced by osteoblasts isolated from null mice. We then focused our studies on the osteoclast, the cell type responsible for bone resorption. Using immuno- florescence, we determine that CNO protein co-localizes with LAMP-2, a lysosome marker protein, in RAW264.7 cells that had been differentiated into osteoclasts with RANKL. By histomorphometry we observed a significant increase in osteoclast number in the distal femur at 16 weeks of age in the cno/cno mice, but this was not associated with changes in bone formation rate. In summary, our data suggest a role for the CNO protein in basic osteoblast and osteoclast physiology. In vitro, osteoblasts lacking the CNO protein unexpectedly had an increased mineralizing ability whereas in vivo, mice lacking CNO protein exhibit an increased osteoclast phenotype, and exhibit an increase in osteoclast number. Thus, CNO may have a role in coupling bone formation with bone resorption.


Juvenile hydrocephalus, the accumulation of cerebrospinal fluid (CSF) in the ventricles of the brain, causes significant morbidity among human children affecting roughly 1 in 500 newborns. The disease manifests due to overproduction, decreased absorption, or restricted flow of CSF. Few genetic causes of this disease are known, and therefore animal models can prove beneficial in identifying candidate genes and studying relevant pathways. The Juvenile hydrocephalus (Jh) mouse line, a lacZ transgene integration on mouse chromosome 9. Homozygous Jh mice exhibit hydrocephalus by two weeks of age and few survive beyond eight weeks. Histological analysis showed a patent aqueduct with no overt brain malformation, indicating communicating hydrocephalus. Scanning electron microscopy found reduced density and loss of orientation of ventricular ependymal cilia, which may be involved in the development of the hydrocephalus. This phenotype represents a novel cause of the disease since no known hydrocephalus mutations map to this genomic region. Analysis of the integration site showed disruption of an uncharacterized gene, 4931429I11Rik, here called I11. The lacZ transgene is expressed in pineal gland, hypothalamus, neuroepithelium lining the aqueduct of Sylvius and choroid plexus, suggesting the expression pattern of I11. The predicted protein product of I11 has no recognizable functional domains, however, and its role in CSF maintenance is unknown. Future research will focus on definitively establishing the role of I11 in hydrocephalus and dissecting its normal function.

Nucleoredoxin Influences Hematopoietic Stem Cell Function Through Wnt Signaling. Bernard A. Ayanga, Karen Mitchell, Melissa Boles, Monica Justice. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Mouse mutagenesis with N-ethyl-N-nitrosourea (ENU) reveals gene functions unique to mammals, and many mutants model human diseases. To examine the function of genes conserved between the mouse and human, a high-efficiency ENU mutagenesis screen using a balancer chromosome was targeted to mouse Chromosome 11. This screen identified Nucleoredoxin (Nxn). Nxxd3/Jhd3 mice have many blood defects in lymphoid and myeloid lineages. However, mice that live long enough to analyze blood parameters were rare in this allele. When HSCs were challenged in heterozygotes, neutrophils were overproduced and increased proliferation of myeloid colonies was observed in vitro. We were not able to examine homozygotes due to their perinatal death. Thus, further analysis of the role of Nxn in the blood could only be carried out in a conditional loss of function allele. The knockout first Nxn allele was obtained from the European Conditional Mouse Mutagenesis Program (EUCOMM), and transmitted to the germline. We currently have crossed them to FLPe deleter mice to remove the sa-ggeo-pA cassette, producing mice with floxed exon 2. We are crossing these mice to vav-Cre and Myxovirus Resistance 1-(Mx1)-Cre in order to further examine the role of Nxn in hematopoiesis.

Identification of Quantitative Trait Loci Controlling Molecular Morphology in a Mouse Model of Human Colorectal Cancer. David J. Bautz, David W. Threadgill. Department of Animal Sciences, North Carolina State University, Raleigh, NC.

Colorectal cancer (CRC) is the second leading cause of cancer death in the United States. Early detection has frequently been cited as contributing to a favorable outcome. However, detection of CRC is impacted by tumor morphology, with the classical polyoid lesions being detected at a much higher rate than flat lesions. Recently, mouse models of CRC that consistently and almost exclusively develop flat lesions have been developed, indicating that specific host genetic modifiers exist that influence tumor morphology. Analysis of the integration site of an uncharacterized gene, 4931429I11Rik, here called I11. The lacZ transgene is expressed in pineal gland, hypothalamus, neuroepithelium lining the aqueduct of Sylvius and choroid plexus, suggesting the expression pattern of I11. The predicted protein product of I11 has no recognizable functional domains, however, and its role in CSF maintenance is unknown. Future research will focus on definitively establishing the role of I11 in hydrocephalus and dissecting its normal function.

A Locus Mapping to Mouse Chromosome 8 Determines Infarct Volume in a Mouse Model of Ischemic Stroke. Christopher L. Bennett, Seehon Keum, Douglas Marchuk. Molecular Genetics and Microbiology, Duke University, Durham, NC.

In an established mouse model of focal cerebral ischemia, infarct volume is highly variable and strain dependent, but the natural genetic determinants remain unknown. To identify these genetic determinants regulating ischemic neuronal damage and to dissect apart the role of individual genes and physiological mechanisms in infarction in mice, we performed quantitative trait locus analysis of surgically induced cerebral infarct volume. After permanent occlusion of the distal middle cerebral artery, infarct volume was determined for 16 inbred strains of mice. Genome-wide linkage analysis was performed for infarct volume as a quantitative trait. Infarct volume varied up to 3-fold between strains, with heritability estimated at 0.88. Overall, 3 quantitative trait loci were identified that modulate infarct volume between B6 and BALB/c. One of these loci mapped to chromosome 8 in a 32nb region with a significant LOD score of 3.2. Subsequent genome wide association studies (GWAS) have narrowed down this region to five candidate genes. Current studies on candidates with significant biological relevance are aimed at identifying strain-dependent expression profiles and targeted exonic sequencing. As well, future studies through the use of zebrafish will be directed towards ascertaining the functional relevance and the pathophysiological consequences of candidate gene knock-down via morpholino antagonism. The identification of the gene in this loci may uncover novel genetic and physiological pathways implicated in the modulation of cerebral infarction and provide new targets for therapeutic intervention in ischemic stroke, and even further in other ischemic diseases.

108C

POSTER: Mouse models of human disease

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

109A

Mouse models of human disease

Christopher L. Bennett, Seehon Keum, Douglas Marchuk.

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The identification of the specific factors for development of therapeutic interventions.
The generation of mouse mutants using conventional knock out approach is a powerful tool to study the role of specific genes. However, this technology shows two major limitations: (i) disruption of many genes result in lethal phenotypes (ii) it does not allow site specific inactivation of a gene. The conditional knock-out strategy overcomes these limitations. When such "floxed" mice are bred with transgenic mice expressing the Cre recombinase in a tissue/cell-specific manner, the gene of interest is knocked out only in that particular tissue or cell type. An added sophistication is the inclusion of temporal control, which can be achieved using a ligand-activated chimeric recombinase, composed of the fusion of the Cre recombinase with the ligand binding domain of a mutated form of the estrogen receptor (ER), which can only be activated by synthetic ER ligands (e.g. tamoxifen, Cre-ERT2, Indra et al. 1999). Large-scale international mouse mutagenesis programs (EUCOMM, NorCOMM and KOMP) are providing conditional knock-out of most mouse genes. As these lines are becoming available to the whole scientific community, the need of a large variety of cell specific deletor lines seems essential. At the ICS, we have generated about 50 Cre transgenic mouse lines expected to express the tamoxifen inducible CreERT2 recombinase in different target tissues and cells. These include different neuronal populations, immune system, adipose tissue, different cell populations in the digestive tract, pancreas, muscle, bone, immune system, reproductive tract, skin. Characterization of the efficacy and specificity of these lines is under way. They are available to the research community and will be a powerful tool for the study of disease genes function, the creation of disease models and to answer questions on the cell/organ autonomous or not character of various pathological phenotypes. For details, see http://www.ics-mci.fr/mousecre/.

Discovering mouse genotypes that share an autistic-like phenotype. Judith A. Blake, Elissa J. Chesler, Jeremy J. Jay, Terrence F. Meehan. Mouse Genome Informatics, Bar Harbor, ME.

Autism spectrum disorders (ASD) represent a group of developmental disabilities with a strong genetic basis. The laboratory mouse is increasingly used as a model organism for ASD and MGI, the Mouse Genome Informatics resource, is the primary model organism database for the laboratory mouse. MGI uses the Mammalian Phenotype (MP) ontology to describe mouse models of human diseases including eight models of mouse ASD. As MGI has over 12,000 genotype-to-phenotype annotations to MP terms that describe aspects of abnormal behavior, we asked the question as to whether other mouse models relevant to ASD research existed in our database. By using the bioinformatics tools VLAD (Visual Annotation Display term enrichment tool) and the Ontological Discovery Environment, we developed an integrative analysis that identifies mouse genotypes described in MGI that share aspects of an autistic-like phenotype and that overlap with previously described ASD mouse models. The genes involved in these genotypes have significant overlap with orthologous human genes associated with ASD as well as many novel genes not previously implicated with the disorder. Prediction of autistic mutant mouse models assists researchers in studying the complex nature of ASD and provides a generalizable approach to find mouse models for other complex human genetic disorders.

Haploinsufficient Role for Pax2/Emx2 in Vesicoureteral Reflux and Other CAKUT-like Malformations. Sami K. Bouali1, Yaned Gaitan1, Inga Murawski2, Robert Nadon3, Indra R. Gupta4, Maxime Bouchard5. 1) McGill Goodman Cancer Research Centre, Montreal, Canada; 2) Department of Pediatrics and Department of Human Genetics, McGill University, Montreal, Canada; 3) McGill University and Genome Quebec Innovation Centre, McGill University.

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of chronic kidney disease. This disease group includes a spectrum of urinary tract defects including vesicoureteral reflux (VUR), duplex kidneys and other developmental defects that can be found alone or in combination. To identify new regulators of CAKUT, we tested the genetic cooperativity between several known developmental regulators of urogenital system in mice. We found a high incidence of urinary tract anomalies in Pax2/Emx2 compound heterozygous mice that are not found in single heterozygous mice. Pax2+/--;Emx2+/--; mice harbor duplex systems associated with urinary tract obstruction, bilil rureter and a high penetrance of VUR. Remarkably, most compound heterozygous mice show low intravesical pressure reflux. Early analysis of Pax2+/--;Emx2+/--; embryos identify under budding defects as the primary cause of urinary tract anomaly. We additionally establish Pax2 as a direct regulator of Emx2 expression in the Wolffian duct. Together, these results identify a haploinsufficient combination resulting in CAKUT-like phenotype, including a high sensitivity to vesicoureteral reflux. As both genes are located on human chromosome 10q, which is lost in a proportion of VUR patients, these findings may help understand the quality of life for individuals with DS.
POSTER: Mouse models of human disease

Abstract number above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

119B

Improved Resolution across the Bbaa2 Mouse Arthritis Susceptibility Locus by SNP Based Genotyping. Kenneth K. C. Bramwell1, Ying Ma1, John H. Weis1, Cory Teuscher1, Janis J. Weis1. 1) University of Utah; 2) University of Vermont.

Abstract: Lyme arthritis is caused by the tick-borne spirochete Borrelia burgdorferi. 30% of patients not treated at the time of the tick bite develop acute arthritis, although serology indicates that others become infected without developing arthritis. Intercrosses between severely and mildly affected mice identified Quantitative Trait Loci on five chromosomes, including Bbaa2 on Chromosome 5 with a LOD score of 10.2. Congenic mapping with recombinant Interval Specific Congenic Lines has narrowed the physical boundaries of Bbaa2. Microsatellite marker availability became a limiting factor to narrowing the Bbaa2 interval. To improve resolution, three Polymerase Chain Reaction (PCR) based genotyping methods for Single Nucleotide Polymorphisms (SNPs) were tested.

Purpose: Improve resolution across the Bbaa2 interval by adopting a SNP-based genotyping strategy.

Methods: Amplification Refractory Mutation System (ARMS)-PCR uses two inner SNP specific and two outer primers. PCR was performed on a PTC-200 Thermal Cycler, and analyzed by Gel Electrophoresis. Small Amplicon High Resolution Melting (HRM) uses two primers surrounding the SNP of interest, producing an amplicon of 50-70 bp. Blocked Probe HRM uses a 3’ Phosphorylated oligo probe overlapping the SNP of interest, and two outer primers producing an amplicon of 65-150 bp. Both HRM methods used LCGreen Plus reagent, and were performed on an LC480 platform.

Results: ARMS-PCR was not time nor cost efficient. Only 10% (1/10) of ARMS-PCR based SNP assays worked, even after optimization efforts. Small Amplicon HRM was the most cost effective methodology. Assay design was straightforward but inflexible due to the small amplicon size required. 81% (9/11) of assays worked, but some assay interpretations were subjective due to small melting temperature differences. Blocked Probe HRM was both time and cost effective. Assay design was straightforward and allowed primer selection flexibility. Interpretation of results was unequivocal. 71% (41/58) of assays worked. Blocked Probe HRM was adopted for genotyping of Bbaa2 ISCL mice.

120C

The Berlin Fat Mouse line is a model for the metabolite syndrome in humans. Gudrun A. Brockmann, Claudia Hantschel, Christina Neusich, Asja Wagener. Crop and Animal Sciences, Humboldt-Universität, Berlin, Germany.

The Berlin Fat Mouse Inbred (BFMI) line is a model for juvenile obesity with features of the metabolic syndrome. It has been selected for high fatness over several generations and, therefore, harbors natural variations leading to the obese phenotype. A recessive gene defect on chromosome 3 in BFMI mice is responsible for 40% of the obese phenotype. The BFMI line accumulates about 5fold higher fat percentage in comparison to the unselected mouse line C57BL/6 (B6) on standard diet. BFMI mice respond to high fat diet with further weight gain. For the characterization of BFMI mice, we measured fat content, energy intake, serum lipids and hormones, and assessed glucose homeostasis in comparison to B6 mice. The main accumulation of body fat in BFMI mice occurs between 6 and 10 weeks and is accompanied by hyperphagia, onset of the fatty liver syndrome and high triglyceride levels compared to B6 mice. Total cholesterol and blood glucose serum levels were similar to B6 mice. At 10 weeks, glucose tolerance was normal, but the insulin level was already increased. At 20 weeks however, glucose clearance was delayed. The endocrine profile of BFMI mice showed increased serum leptin and reduced adiponectin levels compared to B6. The obesity and the endocrine profile of BFMI mice correspond with features of the metabolic syndrome seen in humans indicating this mouse line as an excellent model for humans. We acknowledge support from the BMBF (NGFNplus project 01GS0829) and the Deutsche Forschungsgemeinschaft (GRK 1208).

121A

Cholesterol biosynthesis and metabolism is dysregulated in a mouse model of Rett Syndrome. Hannah M. Brown, Christie M. Buchovec, Monica J. Justice. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Rett Syndrome is an X-linked, neurological and developmental disorder almost exclusively occurring in females. Clinical features of the syndrome include initial language and motor milestone regression, autistic like behaviour, abnormal hand movements and breathing irregularities. Rett Syndrome arises from mutations in the Mecp2 gene, located on the X chromosome. Mouse models with mutations and deletion of Mecp2 have allowed us to begin to elucidate the underlying biology behind this complex syndrome. Most recently, we identified that mice null for Mecp2 have significant dysregulation of the cholesterol biosynthesis, cholesterol metabolism and fatty acid biosynthesis pathways. Cholesterol is an extremely important biological molecule. As well as being a precursor for the synthesis of steroid hormones, bile acids and Vitamin D, cholesterol is a critical component of cell membranes. It is estimated that the half the dry weight of the brain is composed of lipids, accounting for approximately 20% of the body's total cholesterol and is involved in membrane trafficking, signal transduction, myelin formation and synaptogenesis. Following isolation of hippocampus, cerebellum, brain stem and cortex, real time RT-PCR was used to analyse gene expression of enzymes involved in cholesterol synthesis and metabolism, whilst derivatives and metabolites were analyzed using EIA, RIA and tandem mass spec. We identified significant decreases in Scle, Lsx and Sc4md4 as well as a number of other genes involved in cholesterol biosynthesis. Members of the sterol hormone biosynthesis pathway including Aromatase and Cyp11a1 were also significantly decreased. Fatty acid biosynthesis was also dysregulated in Mecp2 null mice. Interestingly, these pathways have been demonstrated to be dysregulated in other neurological disorders including Alzheimer’s and Huntington's. Improved understanding of the requirement for cholesterol biosynthesis in the brain and its involvement in the etiology of Rett Syndrome will lead to the development of novel therapeutic strategies and improved treatment for these patients.

122B

A SOD1 humanising knock-in mouse model of ALS with conditional point mutation. Rosie K. A. Benton-Stanyszyn1, Ann Devoy1, Victor L. J. Tybuliewicz2, Elizabeth M. C. Fisher1. 1) Department of Neurodegenerative Disease, Institute of Neurology, UCL, London; 2) University of Western Australia,; 2) MRC National Institute for Medical Research, London, United Kingdom.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease involving loss of upper and lower motor neurons. Death, due to respiratory failure, usually occurs within 3 years of diagnosis. Most cases of ALS are sporadic, but ~10% of patients have an inherited form. Around 20% of inherited ALS cases are caused by a mutation in the gene encoding superoxide dismutase 1 (SOD1). In the vast majority of ALS patients, SOD1 mutations are inherited as a single gene. There are currently 12 disease associated SOD1 mutations in published transgenic mouse models. These are basic tools that have been used to elucidate the pathological mechanisms of ALS, producing results pointing to an involvement of multiple pathways in the disease’s pathogenesis, including misfolded mutant SOD1 triggering aberrant mitochondrial function, endoplasmic reticulum stress pathways or axonal transport defects. There are however, discrepancies between the phenotype observed in mouse models and the human disease. These may partly arise from issues of protein load due to models bearing multiple copies of the gene under exogenous control resulting in increased SOD1 protein expression. In order to better represent the human disease, we are aiming to create a new SOD1 mouse model. A knock-in (KI) gene targeting strategy will be used, replacing the mouse orthologue Sod1 with human SOD1 and introducing disease associated point mutations. This model will have a conditional point mutation which can be turned on or off. BAC recombineering will be used to make the construct. This new SOD1 KI will be under the control of the endogenous mouse promoters and so will be expressed at endogenous levels in the appropriate tissues. We intend to produce a more robust model of the human disease, with fewer artefacts caused by the construct. It is hoped that it will provide significant insight into the development of ALS and what treatments may be required to halt or slow disease progression.

122C

A genetic approach to α-synuclein function. Deborah E. Cabin, Megan Casey. McLaughlin Research Institute, Great Falls, MT.

Parkinson's disease (PD) is the second most common neurodegenerative disease in humans. The common sporadic form of the disease is of unknown etiology, but rare familial forms are known. The first gene linked to a familial form of PD was α-synuclein (SNCA), encoding a small, natively unfolded, pre-synaptic and nuclear protein. This protein is also linked to sporadic PD as it is a major component of Lewy bodies, intracellular inclusions that are the pathologic hallmark of the disease. These inclusions are probably an attempt by the cell to sequester SNCA rather than being toxic themselves. However, it is possible that sequestration of SNCA in Lewy bodies prevents the protein from performing some function that is important under stress conditions. The normal functions of SNCA are not well understood; mice lacking SNCA have some subtle synaptic phenotypes, but are healthy and live a normal lifespan. SNCA's normal functions must be better defined before we can evaluate their contributions to PD. We have taken a genetic approach to understand SNCA's normal functions, a sensitized ENU mutagenesis screen using mice that lack SNCA. The goal is to find mutations that cause a more severe phenotype in the absence of Snca to identify pathways in which SNCA is required. This approach has the advantage of being unbiased by the protein's subcellular localization or physical properties.

We have finished first-pass screening of 125 pedigrees for recessive mutations; 8 mutant lines are being bred further to determine if the mutations are indeed sensitized. One sensitized mutation has been confirmed, a missense mutation in Snca that sensitizes the protein to Atp7a. Atp7a encodes a trans-Golgi copper transporter. While males carrying the mutation die regardless of SNCA status, mutant females deleted for Snca have a statistically significant higher rate of early death (<35 days) than mutant females that are wild type or heterozygous for Snca. Immunohistochemistry using male brains indicates that Snca may play a role in proper localization of Atp7a during development.

124A

Combined use of a ROSA26-lox-STOP-lox-Pdm14 knock-in mouse model and intraducal injection of a self-deleting lentiviral Cre recombinase for the modeling of
tumor initiation in breast cancer. Brandi L. Carofino1,2, Monca J. Justice1. 1) Translational Biology and Molecular Medicine; 2) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Genetically engineered mouse models represent powerful tools for studying breast cancer initiation and progression. The lack of mammmary-specific promoters, however, has proved to be a major challenge in the field. Currently, transgenes are expressed from the mouse mammary tumor virus long terminal repeat (MMTV-LTR) and whey acidic protein (Wap) promoters. Individual lines vary in expression pattern and level depending on the site of integration, and none are entirely mammary specific. Also, MMTV and Wap promoter-driven expression is found throughout the ductal tree and is regulated by lactogenic hormones, not recapitulating human disease initiation, which is likely driven from a small number of cells.

We developed a novel mouse model to study breast cancer initiation, circumventing the problems associated with MMTV and Wap-driven transgenes. We generated a ROSA26-targeting vector containing a lox-STOP-lox cassette upstream of our chosen oncogene (Prdm14) and an IRES-EFIP marker. When knock-in to the ROSA26 locus, this construct allows for spatial and temporal Cre-mediated excision of the STOP cassette and activation of exonspecif Cre. Utilizing an intraductal injection of a self-deleting lentiviral Cre recombinase will have several advantages: 1) self-deletion will reduce toxicities associated with sustained Cre expression, 2) injection will ensure mammmary-specific expression and will reduce animal production time/costs, 3) timing/titer of lentiviral injection will control the type and number of cells expressing the oncogene and 4) ROSA26-driven oncogene expression is predictable, and not subject to position-effect variegation that leads to transgene silencing. We will use this system to determine if Prdm14 expression in a limited number of mammary cells is sufficient to drive tumorigenesis and will use the lentiviral footprint and EGFP marker to establish tumour clonemogeneity and transplantability.

125B

The influence of α-synuclein on AJ plaque formation in TgCRND8 mice. MegAnne Casey, Jessica Bialczak, Deborah Cabin. McLaughlin Research Institute, Great Falls, MT.

Parkinson’s disease (PD) and Alzheimer’s disease (AD) are the two most common neurodegenerative disorders in humans. Pathologically, overlapping characteristics of pure PD and AD are found in dementia with Lewy bodies (DLB) and the Lewy body variant of AD. Lewy bodies are deposits composed mainly of the misfolded protein, α-synuclein (SNCA), and are also the hallmark pathology of PD. Over a decade ago, a fragment of SNCA was found to be the non-amyloid component of amyloid β plaques. Amyloid β plaques are made of the Aβ-peptide (Aβ), a fragment of amyloid precursor protein (APP), and are part of the definitive pathology of AD patients. These observations have long suggested a relationship between SNCA and the Aβ-peptide. To identify the potential pathologic interaction between these proteins, researchers crossed an APP transgene onto a Snca knockout background. This allowed them to quantitate Aβ plaque deposition. Kallhoff et al. (2007) reported that cortical Aβ plaque accumulation in Tg2576 mice on the Snca null background was significantly higher (3-4 fold) than in the Tg2576 wild-type (WT) controls at 18 months of age. To develop a more tractable model, we have bred a transgenic mouse expressing a fragment of APP (AP605 (TgCRND8)) onto our Snca null mouse; use of this transgene allows us to study the influence of APP on Aβ plaque accumulation at 3 months vs. 9 months for Tg2576. We have also extended the experimental design to include human A53T mutant and WT SNCA PAC transgenes in combination with TgCRND8 in mice lacking endogenous Snca. Preliminary results indicate that the reported increase in Aβ plaque load in the absence of Snca is not replicated using TgCRND8 on the 129Psic genetic background. Complete analysis will allow us to compare the influences of mouse and human SNCA on Aβ plaque accumulation in the TgCRND8 mice. We are also investigating physical localization of the SNCA species in relationship to Aβ plaques by confocal microscopy, and can predict Aβ plaque localization by adjacent SNCA accumulation.

126C

Compensatory Transcriptome Analyses in Homozygous KOMP Knockout Mice. W. Chen1,2, W. Willis1, A. Cipolle1, E. Engelhard1, K. Drake2, K. Lloyd2, D. West1,2. 1) Mouse Biology Program, University of California, Davis, CA; 2) Serologix Inc., Austin TX; 3) Childrens Hospital of Oakland Research Institute, Oakland, CA.

For the KOMP-Phenotyping Project Pilot (www.kompphenotype.org ) we are using compensatory transcriptome analyses (illumina beadarray) to predict gene function in homozygous (HOM) mutant mice. Tissue selection is informed by published gene surveys and LacZ reporter expression. We have recently completed these analyses in the Leprdb/db mutant for which there is substantial pathology, and in two Riken clone KOMP mutants of unknown function: 1300002K09Rik and 170002913Rik. For each mutant, we used at least 2 replicates for each tissue and compared the expression profiles against C57BL/6N profiles. Criterion for differential expression was 2-fold change relative to control. Hits were mapped to pathways using KEGG (www.genome.jp/kegg/) and proprietary software from Serologix using a Bayesian approach (www.serologix.com/ ). For d/db mice, 235 genes were up- and 185 were down-regulated in 4 different tissues. 14 of the 15 pathways affected correlate with known physiological phenotypes in leprdb/db mice. Network Analysis found 7 related pathways each affected in 3 different tissues, 4 of which are relevant to published physiological phenotypes in leprdb/db. For 1300002K09Rik KO, 171 genes were up- and 76 were down-regulated in 3 different tissues. CytoE4 was up regulated more than 6-fold and hit 4 pathways (retino metabolism, PPAR signaling, arachidonic acid metabolism and fatty acid metabolism) with 3 other genes also affected in these pathways. For 170002913Rik KO mice, 212 genes were up- and 396 were down-regulated in 4 different tissues. The most affected pathways in 170002913Rik were drug & xenobiotic metabolism, arachidonic acid metabolism, vascular smooth muscle contraction and PPAR signaling, with at least 6-fold differences in expression levels. These data illustrate the compensatory transcriptome analyses for predicting gene function. Supported by NIH Grants: U01HG004080-04S1, 3U42RR024244-03S2.

127A


Advanced intercrossed lines (AILs) are an ideal resource for fine-scale quantitative trait loci (QTL) mapping. However, analysis of AIL data requires appropriately incorporating the information of complex relationships among individuals into the statistical model, which can lead to testing and computational issues. We developed a computational strategy that takes advantages of both top-down and bottom-up approaches and an R package to calculate identity coefficients for around 700 F34 mice in a pedigree of around 6000 individuals. We used this tool to study mouse SNPs for which gene localization is unknown. We found significant linkage between two SNPs in the exon of the enolase 1 gene and 30 genes with potential functional implications. We also used this tool to map QTLs. For each mutant, we used the appropriate analysis by using permutation-based tests to control for false discovery rate. Based on the results of this analysis, we will continue to study mouse SNPs with potential functional implications using this package and will be able to use the results to design follow-up experiments. This package is available at GitHub (https://github.com/rianycheng/AILQTL).

128B

Genetic dissection of resistance to Yersinia pestis of SEG/Pas mice. Lucie Chevallier1, Charlène Blanchet1, Jean Jaubert1, Emilia Pachulce2, Christian Demere3, Claudia Pommerenke1, Jean-Marc Cavaillo1, Klaus Schughart4, Elisabeth Carmiel2, Jean-Jacques Panthier1, Xavier Montagutelli1. 1) CNRS URA 2578, Mouse Functional Genetics, Institut Pasteur, Paris, France; 2) Yersinia Unit, Institut Pasteur; 3) Cytokines and Inflammation Unit, Institut Pasteur; 4) Department of Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany.

Plague is a deadly infectious caused by Yersinia pestis, a highly pathogenic gram-negative bacterium. While antibiotics are efficient against Y. pestis, the appearance of a multi-resistant strain highlights the need for new strategies. We showed that SEG/Pas mice, derived from Mus spretus, are resistant to a subcutaneous injection of 100 Y. pestis bacteria, whereas all classical laboratory strains tested are susceptible. A backcross between SEG/Pas and C57BL/6 mice strains led us to identify three quantitative trait loci (QTL) controlling the survival rate on Chromosomes 3, 4 and 6. The three QTLs were shown to contribute to the resistance against Y. pestis in an additive manner. We produced three congeneric strains carrying SEG alleles at each of the three QTLs in a C57BL/6 background. Bi- and tri-congeneric strains were also created by crossing congens. Survival and time to death were characterized for each of these strains. Our results indicate that the males and females triple congens survive to about 32% while all C57BL/6 die. Since this is much less than the survival rate observed in the backcrosses heterozygous at the three QTLs, other regions must play a role in resistance of SEG mice against Y. pestis. In parallel to genetic studies, we determined that SEG/Pas macrophages exhibit distinct phenotypes compared to C57BL/6 macrophages upon in vitro exposure to Y. pestis. Gene expression differences between SEG/Pas and C57BL/6 macrophages in response to Y. pestis infection are investigated using microarrays. Coupling QTL mapping with transcriptome analysis and data from congeneric strains will enhance our ability to unravel the mechanisms involved in resistance to plague in SEG/Pas.

129C

Functional characterization of three loci (Ses1, Ses4 and Ses5). Marie Cheveon1,2, Mayas Naccache1, Megan Eva1, Danielle Malo1,2,3. 1) Experimental Medicine; 2) Human Genetics; 3) Complex Traits Group, McGill University, Montreal, QC, Canada; 4) P3G, Montreal, QC, Canada.

Selenium deficiency and S. Enteritidis caused a food-borne disease in mice. To study the persistence of Salmonella during the late phase of infection, a mouse model was developed using C57BL/6 (B6) mice that clear the bacteria completely from the spleen and lymph nodes within 42 days post-infection and 129Psic/EVTac (129Psic) mice that become chronic carriers. Linkage analyses using a cross between B6 and 129Psic mice led to the mapping of ten quantitative trait loci, Ses1 (Salmonella)
Enteritidis susceptibility locus 1) to Ser10, associated with high bacterial load in 129S6 mice. In the females, both Ser1 and Ser3 showed significant effects on bacterial clearance and two significant interactions between Ser1 and Ser4 and between Ser1 and Ser5 accounted for 72% of the total phenotypic variation. We have created congenic mouse strains by introgressing specific B6 chromosomal regions onto 129S6 background for Ser1, Ser3, Ser4 and Ser5. Phenotypic analysis confirmed that Ser1 contributes to bacterial clearance in both sexes. Ser1 was shown to be the gene underlying Ser1 using Slc11a1-deficient mice. In this model, 129S6-Slc11a1+/- mice have a significantly lower bacterial load compared to 129S6 mice and they mounted an earlier and more vigorous T-cell response compared to a strong Tcell response in 129S6 mice. To functionally validate in vivo the interaction between Ser1 and Ser4 or Ser5, we created double congenic mice. Bacterial counts in the spleen and the liver demonstrated that both the 129S6.B6-Ser11Xser4 and the 129S6.B6-Ser1Xser5 mice clear S. Enteritidis more efficiently than 129S6 or single congenic mice at day 42 post-infection validating the interaction terms identified by statistical analyses. In summary we have shown in this model that many loci with moderate to small effects caused variation in the trait and that context dependent effects were identified as significant genotype-by-genotype and genotype-by-sex interactions.

130A The genetic relationship between obesity and long bone cross-sectional morphology in the LG/J by SM/J intercross. James M. Cheverud1, Jane Kenney-Hunt1, Hatti Hylery, Connie Shao2, Lorri Leung1, Linda Sandell2, Matthew Silvia1. 1) Dept Anatomy/Neurobio, Sch Med, Washington Univ, St Louis, MO; 2) Dept Orthopedic Surgery, Sch Med, Washington Univ, St Louis, MO. Recently several different mechanisms have been proposed linking obesity and osseous development in mice, including the mechanical effects of excess weight, direct leptin action and the effects of leptin mediated by the central and sympathetic nervous systems, and the effects of bone-derived calcitonin on energy regulation and adiposity. Here we evaluate the genetic relationship between obesity and long bone cross-sectional morphology in the advanced intercross of LG/J and SM/J mice (Wustl:LG,SM-G34). The population contains 1169 animals in 137 full-sib families for an average litter size of 8.5 pups per family. Animals were reared by their mothers for three weeks after which half of each litter was placed on a high or low fat diet (Cheverud et al., 2004). The diets are isocaloric but the low fat diet has 15% of its calories from fat while the high fat diet has 42% of its calories from fat. The sample is composed of 282 low fat-fed females, 292 high fat-fed females, 279 low fat-fed males and 286 high fat-fed males. Males have 11% more cortical bone than females and animals on a high fat diet have 3% more cortical bone than those reared on a low fat diet. Heritabilities of bone cross-sectional properties are moderate (~57%). Genetic correlations between bone and obesity are low to moderate (rG = 0.3 – 0.6). We mapped 50 different QTLs of which 3 were also significant for fat.

131B Uncovering Novel Therapeutic Targets Promoting Neuroprotection Against Oxidative Stress. Kyoung-in Cho, Haiqing Yi, Paulo Ferreira. Ophthalmology, Duke University Medical Center, Durham, NC. Identification of factors modulating oxidative stress may uncover therapeutic targets to unmet clinical needs. Oxidative stress elicited by light and the parkinsonian neurotoxin, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is a deleterious risk factor linked to neurodegenerative diseases, such as age-related macular degeneration and Parkinson's.

132C INVESTIGATION OF MYOTILIN AS A MODIFIER GENE IN A MOUSE MODEL OF CARDIO Myopathy. Pei-Lun Chu1,2, Lan Mao3, Howard Rockman1, Olli Carpen4, Douglas Marchuk1,2. 1) Program in Genetics and Genomics, Duke University, Durham, NC, USA; 2) Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA; 3) Department of Pathology and Neuroscience Program, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland. In striated muscle, the Z-disc of the sarcomere functions as a stretch-sensing interpretor and a scaffold for sarcomeric structural proteins. Myotilin (MYOT), a Z-disc structural protein in skeletal and cardiac muscle, is important in force sensing and transmission in the sarcomere. Missense mutations in human myotilin cause clinical phenotypes that primarily affect skeletal muscle, but some patients also develop cardiomyopathy. Because of its potential biological relevance in cardiac function, we hypothesized that MYOT plays a role in cardiomyopathy. Although the Z-disc is a major component of the sarcomere, myotilin knockout (KO) mice have a normal life span and do not show either skeletal myopathy or an overt cardiac phenotype. However, many mouse knockouts of Z-disc proteins do not exhibit a phenotype except under stressed conditions. Therefore, we investigated the role of the myotilin in cardiomyopathy in Myot KO mice using different stressors. Under acute, isoproterenol-induced stress, the Myot KO mice showed similar cardiac function as wild type (WT) animals. However, under chronic stress using a pressure-overload model (transaortic constriction), the Myot KO mice preserve much of their cardiac function, while WT animals exhibit extreme cardiomyopathy. This suggests that the Myot function is protective in chronically stressed conditions such as might be found in heart failure. We are currently investigating the underlying molecular mechanism of this protective effect.

133A Post-translational regulation of Lunatic fringe is critical for function of segmentation clock during somitogenesis. Susan E. Cole, Dustin R. Williams, Emily T. Shifley. Molecular Genetics, The Ohio State University, Columbus, OH. Somites are the embryonic precursors of the vertebrae, ribs, and skeletal muscles. They form from the presomitic mesoderm by a periodic process called somitogenesis. Perturbations of somitogenesis in humans can cause congenital defects including spondylocostal dysostosis and kyphosis. Somitogenesis is controlled by a segmentation clock that governs periodic transcription of segmentation clock genes. Post-translational regulation of Lunatic fringe is critical for function of the segmentation clock, the cyclic transcription of Lfng must be coupled with post-transcriptional mechanisms that confer a short protein half-life. Lfng protein acts cell-autonomously in the Golgi, but we have found that the Lfng protein is processed by SPC proteases, and is then released into the extracellular space. We hypothesized that this Lfng protein processing promotes the secretion and inactivation of the Lfng protein, rapidly terminating Lfng activity during the "off" stages of the clock. To test this functional relevance of this processing we created a novel targeted allele of Lfng that tethered the protein in the Golgi, preventing postprocessing and secretion, without affecting transcriptional control or splicing. This allele is predicted to create a hyperactive fringe protein with dominant effects due to its increased functional half-life. As predicted, mice heterozygous for the tethered Lfng allele exhibit severe segmentation defects, malformed ribs and vertebrae, and truncated tails. Production of epithelial somites is abnormal in heterozygous embryos, and rostral-caudal patterning of somite derivatives is perturbed. Further, segmentation clock function is altered during somitogenesis. These findings highlight the importance of Lfng protein processing and turnover during somitogenesis, and support a novel post-translational mechanism allowing tight temporal and spatial modulation of Notch signaling during somitogenesis. This novel allele of Lfng can be used to examine the functions of Lfng during other developmental decisions.

134B c-Fos Induction Associated With Ethanol Withdrawal In Chromosomal Component 1 Congenic and Girk3 Knockout Mice. Alexandre M. Colville1, Gang Chen1, Laura Kozell2,3, Kari Buck1,2. 1) Behavioral Neuroscience, OHSU, Portland, OR; 2) Portland Alcohol Research Center, VA Medical Center, Portland, OR. Our goal is to dissect the new and molecular substrates by which quantitative trait loci (QTLs) influence ethanol dependence and associated withdrawal. Using mapping populations derived from DBA/2J (severe Eioh withdrawal phenotype) and C57BL/6J (mild Eioh withdrawal phenotype) mice, we previously identified QTLs on distal Chromosome 1 (Chr 1) with large effects on ethanol withdrawal. We created congenic strains with segment 1 and found that mice were more susceptible to acute ethanol withdrawal. We created a congenic strain on chromosome of B6 strain superimposed on a genetic background that is >98% from the D2 strain. Genetic noise from the remainder of the genome is nearly eliminated, which allows for comparisons of the neural circuitry between the congenic and background strains. Fine-mapping to a 0.44 Mb interval and detailed molecular analyses of the genes within this interval identified Kcnf1 (which encodes Girk3,
a subunit of G-protein-dependent inwardly-rectifying K+ channels) as a high-quality candidate gene for QTLs affecting withdrawal from ethanol, zolpidem and pentobarbital (J Neurosci 29(1662, 2009)). c-Fos expression was used as a marker for neuronal activation to compare congenic and background strain mice at peak ethanol withdrawal and control animals administered saline. Our results revealed significant strain x treatment interactions (p<0.05) in the prelimbic and cingulate cortices, amygdala, nucleus accumbens shell, and substantia nigra pars reticulata. In addition, trends (p<0.1) were seen in the nucleus accumbens core and ventral pallidum. Preliminary data using GIRK3 knockout mice also indicate less ethanol withdrawal associated activation compared to wildtype mice in regions that include the prelimbic cortex and amygdala. We conclude that Chromosome 5 QTLs on withdrawal may involve the amygdala and prelimbic cortex, where Kcnj9 mRNA and GIRK3 protein are abundant. Future studies will test the role of the neural and genetic targets identified in ethanol physiological dependence and associated withdrawal.

135C Association mapping of endurance exercise capacity in 32 inbred mouse strains. Sean M. Courtney, Michael P. Massett. Texas A&M University, College Station, TX.

Endurance exercise capacity varies across strains of inbred mice suggesting that genetic variation contributes to this phenotype. However, endurance exercise capacity has only been measured in a limited number of mouse strains. Therefore, the purpose of this study was to increase genetic diversity by measuring endurance exercise capacity in 32 strains of inbred mice and to identify genetic determinants of endurance exercise capacity. Endurance exercise capacity, defined as run time, work performed and distance run was assessed in male mice (n=6) from 32 inbred strains using a graded treadmill endurance exercise test. To identify QTL, we employed genome wide association mapping using an efficient mixed model association (EMMA) algorithm. Exercise capacity was significantly different across strains. Run time varied by 2-fold between the high running strain (AKR/J) and the low running strain (AJ). These same strains showed a 15- fold difference when exercise capacity was expressed as work performed. Distance was also significantly different across inbred strains with distance run being 16-fold higher in CAST/EiJ mice compared with A/J mice. Significant associations (P<0.05) were identified for all traits. QTL for run time were identified on Chromosomes 2, 3, 4, 9, 11, 13 and X. QTL for running distance on Chromosomes 1, 8, 10, 11 and 16. Two significant associations on Chromosome 1 (~26 Mb and ~70 Mb) were common to all three traits. Common associations for work and distance were found on chromosomes 1 (~3 Mb), 10 (~19 Mb), and 16 (~66 Mb). Our data significantly increase the number of strains utilized to assess exercise capacity and identify several putative QTL for further study of the genetic basis for exercise capacity. Supported by NIH grant HL085918 to MPM.

136A Rwhs, an ENU-induced mouse model for Bochdalek congenital diaphragmatic hernia in humans, reveals the complexity of the genome. Sally H. Cross, Lisa McKie, Ian J. Jackson. MRC Human Genetics Unit, Edinburgh, Lothian, United Kingdom.

Retinal when the ENU-induced mutation that causes white spots on the retina when heterozygous but is lethal when homozygous. Homozygous embryos have pulmonary hypoplasia and Bochdalek congenital diaphragmatic hernia. This malformation is present in 1/2500 live human births, and even with corrective surgery a mortality of ~30%. Currently, there is no mouse model for this condition. Rwhs maps to a small interval on Chromosome 11 containing Tmem98 and part of the Myelid gene. Sequencing of the entire interval identified one nucleotide substitution which changes a highly-conserved isoleucine to threonine (I135T) in Rwhs, a novel ubiquitously-expressed gene predicted to encode a transmembrane protein. A targeted loss-of-function allele of Tmem98 and another ENU-induced missense mutant, M1L, are homozygous lethal and cause mid-line body wall closure defects. Both complement Rwhs but do not complement each other. A BAC containing Tmem98 can rescue the lethality of the M1L allele but does not rescue the Rwhs recessive lethal phenotype. A BAC recombinant to contain the I135T amino acid substitution can also rescue M1L lethality. All these results suggest that the lethal diaphragmatic hernia phenotype in Rwhs homozygotes is not caused by a straightforward detrimental effect on Tmem98 function caused by the isoleucine to threonine substitution. The I135T substitution either alters Tmem98 function in an unusual genetically complex way or the underlying nucleotide mutation affects another gene. Either way, the very interesting diaphragmatic hernia phenotype appears to be specific to the Rwhs mutation and would be unlikely to be discovered by any other method than ENU mutagenesis.

137B Two new detected QTL interact with Slc11a1 (formerly Nump1) gene to modulate pristane-induced arthritis in mice. Marcelo De Franco1, Antonella Galvan2, Luciana Peters1, Tatiane Canhamero1, Francisco Vorratto2, Andrea Borrego2, Jose Jensen3, Wafa Cabrera, Nancy Starobinias2, Orlando Ribeiro2, Cristiano Rossato3, Tommaso Dragani4, Olga Ibáñez1, 1Laboratorio de Imunogenética, Instituto Butantan, Sao Paulo, Sao Paulo, Brazil; 2 Department of Experimental Oncology, Istituto Nazionale Tumori, Milan, Italy. 3, 4, Almirax (maximum inflammation) and AlRmim (minimum inflammation) phenotype selected mice showed distinct susceptibility to pristane-induced arthritis (PIA). AlRmimss mice homozygous for Slc11a1 S (gly169asp) non functional allele were more susceptible than the other lines. Slc11a1 S mutation affects macrophage and neutrophil activity. The aim of this work was to identify genes in acute inflammatory reaction loci that interact with Slc11a1 alleles to modulate PIA. Mice received two ip injections of 0.5 ml pristane with 60 days of interval. Global gene expression analysis was performed on Affymetrix mouse 1.0 ST microarrays (27k genes) using individuals mRNA (n=4) of arthritic or control paws. In parallel, genome wide association studies were performed to evidence susceptibility to arthritis QTL in F2 (AlRmim x AlRmim) population. High significant (LODscore > 4) arthritis QTL on chromosomes 5 and 8, and several suggestive ones on chromosomes 7, 17 and 19 were detected. Global gene expression analysis demonstrated significant (P<0.001) over-represented genes related to inflammatory response and chemotaxis in AlRmimss and AlRmimss mice, as well as in AlRmim heterozygous group. Higher up-regulation of chemokines Cxcl1, Cxcl9, Cxcl5, Cxcl13 genes on Chromosome 5 were observed in AlRmimss than in the other lines. In Chromosome 8, macrophage scavenger receptor 1 and heme oxygenase (decycling) 1 genes were also more expressed in AlRmimss mice. qPCR experiments validated microarray analysis. These results revealed two significant arthritis QTL (on Chromosome 5 and 8) interacting with Slc11a1 gene to create a gene expression profile which enhance AlRmimss mice susceptibility to PIA. Financial support: Fapesp and CNPq.

138C Transglutaminase 4 is necessary for the formation of the copulatory plug and normal fertility rate in male mice. Matt D. Dean. Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

After ejaculation, semen coagulates to form what has been referred to as a copulatory plug in a wide variety of animals, including mice. A large body of data supports the hypothesis that the plug evolved to inhibit fertilization by competitor males. It remains unknown whether the plug also plays a role in non-competitive matings, as could occur in the context of male-female signalling. Here we test this alternative hypothesis in two steps. First, we establish a knockout model for transglutaminase 4, a gene that both evolutionary and biochemistry studies have suggested is important for the molecular crosslinking that leads to semen coagulation. With this powerful model, we confirm that this single protein is necessary for copulatory plug formation, as knockout males fail to form a plug. Second, we show that in non-competitive matings, knockout males are effectively sterile. Alliferina females are infertile with wild type in terms of sperm count, motility, and fertility, but are able to fertilize oocytes, as embryos sired by knockout males implant in the female's uterine horns. Failure to implant indicates that the copulatory plug contributes to some minimal stimulatory threshold necessary for pseudopregnancy (whereby the uterine horns are primed for implantation), and/or that the plug affects female choice dynamics.

139A Comprehensive review of a high throughput lacZ reporter gene expression screen. Jeanne Estabel, Elizabeth Tuck, Damian Carragher, Jacqueline K. White, Ramiro Ramirez-Solis, on behalf of the Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, Cambridgeshire, United Kingdom.

The Sanger Mouse Genetics Project (GMGP) aims to make a significant impact on our understanding of the function of genes and their role in disease by generating, characterising and archiving in the order of 200 lines of knockout mice per year. In addition to a standardised battery of primary phenotypic testing, the presence of the β-galactosidase reporter gene. Sequencing of Slc11a1 reporter gene expression screen. Our results revealed a significant impact on our understanding of the function of genes and the role in disease by generating, characterising and archiving a wide variety of knockout mice per year. In addition to a standardised battery of primary phenotypic testing, the presence of the β-galactosidase reporter gene within the targeting construct has proven to be a powerful tool to gain an understanding of gene function by providing translational expression data for endogenous genes. To date, expression patterns (adult and E14.5) in over 220 genes are available (www.sanger.ac.uk/mouseporta). Here we present a comprehensive review of the MGP lacZ expression screen. Specifically, we present a validation of the method through comparison to published expression data, and characterisation of endogenous β-galactosidase staining. We describe how we have enhanced the search potential of our data through annotation with standardized ontologies. We outline the technical improvements we have made, including strategies to tackle staining inconsistencies and penetration issues, and summarize the limitations of this method such as cost, longevity, influence of allele design, reproducibility and the snapshot nature of the data. Finally we present examples linking expression data with phenotypic observations.

140B Variation in Sperm quality of a wild rat fed by the extract of Phyllanthus amarus. HANNAH EDIM ETTA1, ENEOBOEFGFFIOM ENEOBOFGH, PAUL BASSEY UDOH2, BASSEY OKON2. 1 BIOMEDICAL SCIENCE, CROSS RIVER UNIVERSITY OF TECHNOLOGY, CALABAR, NIGERIA; 2 ENVIRONMENTAL BIOLOGICAL SCIENCE.
Host polymorphisms contribute to differential disease phenotypes in response to two respiratory viruses: Influenza A Virus and SARS Coronavirus. Martin T. Ferris, Lisa Gralinski, David L. Aylor, Ryan Buss, Alan C. Whitmoe, Fernando Paro-Manuel de Villena, Ralph S. Baric, Mark T. Heise. 1) Carolina Vaccine Institute, University of North Carolina at Chapel Hill; 2) Department of Genetics, University of North Carolina at Chapel Hill; 3) Department of Microbiology, University of North Carolina at Chapel Hill.

Influenza A Virus (IAV) and SARS Coronavirus (SARS-CoV) are two acute respiratory viruses which cause variable disease severity across the human population. It is likely that host genetic polymorphisms play a role in determining this disease severity, although the acute nature of these viruses makes human population based studies unfeasible. In order to investigate how host genetic variation affected susceptibility to these two diseases, we studied the response of the pre-Collaborative Cross (pre-CC) population to IAV and SARS-CoV infection. We challenged animals from the eight inbred founder strains, as well as 300 pre-CC animals with either mouse adapted IAV strain A/PR/8/34 or mouse adapted SARS-CoV strain MA15 and observed disease course over 4 days. Similar to the human population, pre-CC animals displayed a wide range of disease associated phenotypes (including several disease phenotypes not seen in the founder strains). Although there was significant population-wide variation in disease-associated phenotypes, distinct patterns of population-wide disease variation were seen in the groups infected with IAV as compared to SARS-CoV. Genome wide association studies identified several quantitative trait loci (QTLs) contributing to the host responses to IAV and SARS-CoV. Confirmation of the unique patterns of population-wide disease associations for each of these QTLs was virus specific. These results confirm the utility of using population-based experimental approaches in identifying host genetic polymorphisms contributing to complex viral diseases.


Down syndrome (DS), a complex genetic disorder in humans, is due to the triplication of human chromosome 21 (Hsa21) and results in congenital heart defects in approximately 50% of DS newborns. The Mus musculus Ts65Dn model, with triplication of approximately 132 genetic orthologs to Hsa21, has various phenotypes corresponding to those found in human DS, including neonatal lethality and cardiovascular defects. We expect that dosage imbalance in Ts65Dn embryos causes the misexpression of triplicated genes, thereby disrupting expression of other genes throughout the genome, affecting the observed cardiovascular abnormalities. We utilized proteome analysis to examine the array of proteins expressed at embryonic day E14.5, during the final stages of cardiac septation. Analysis of two-dimensional (2D) protein gels with Delta 2D software followed by tandem mass spectrometry (MS/MS) with Mascot data analysis allowed identification of proteins that are misregulated in the trisomic vs. euploid sibling embryos. Modification of methodologies was critical as very small masses of embryonic heart tissue were the starting material for protein extraction and analysis, and entailed minimizing volumes, eliminating steps, and increasing staining sensitivity. This work allowed identification of nine protein spots, such as cardiac myosin (MLC2a), reticulocalbin, and e-zinc, which showed significantly higher levels of protein in trisomic hearts than in euploid siblings. Analysis of protein spots significantly underexpressed in E14.5 trisomic hearts identified myosin regulatory light chain 2 ventricular isoform (MLC2v). As a critical component of chamber specification MLC2v overexpression could result in abnormal septation; compensatory action by MLC2a may mitigate this effect. This work represents a novel approach to evaluating the effects of dosage imbalance during development and has identified multiple proteins whose abnormal expression levels may disrupt the normal protein milieu during cardiogenesis to produce the pathological conditions seen in trisomic embryos with cardiovascular defects.

High throughput knockout mouse phenotyping: A systematic review of alleles exhibiting metabolic abnormalities. Anna-Karin Gerdin, Jacqueline White, Ramiro Ramirez-Solis, on behalf of the Sanger Mouse Genetics Project. Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom.

The Mouse Genetics Project (MGP) was designed to make a significant contribution to our understanding of mouse as a model organism and its role in disease by generating, characterising and archiving in the order of 200 lines of knockout mice per year. Our phenotyping pipeline aims to perform a broad, yet high throughput, characterisation of the knockout mice. With the growing number of genes analysed and annotated it becomes possible to add value by clustering genes by disease area. Here we select phenodrivers identified from the metabolic screen to assess clustering. A key point to consider is that abnormalities will manifest either as a direct result of the targeted allele or as a consequence of a primary phenotypic observation (a secondary effect). Furthermore, phenotypic outcomes are often dependent, for example, altered body weight will commonly result in altered body composition and/or indirect calorimetry results. Systematic review of in vivo/in vitro (body weight, body composition, indirect calorimetry, glucose tolerance and clinical chemistry) phenotyping results with in silico knowledge (such as GO terms, pfam, interpro domains, protein structure and interaction potential) can be used to identify gene clusters. One obvious group of metabolically interesting genes identified are involved in glucose metabolism. In contrast, an example of a secondary effect metabolic cluster are mutants hyperactive due to an inner ear abnormality. We will present examples of this clustering and a triage based workflow which can be used to pursue interesting alleles for secondary metabolic phenotyping.

ENU mutagenesis identifies mouse mutants with cortical patterning defects resembling human brain malformation syndromes. Yann Herault, Arianna Franca, Erik M. Kelly, Clara S. Moore. Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom.

ENU mutagenesis identifies mouse mutants with cortical patterning defects resembling human brain malformation syndromes, Seungshin H2, Wulf Stottrup, Amy Bernard, David R. Beier. 1) Brigham and Women’s Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA; 3) Allen Institute for Brain Science, Seattle, WA. Formation of a six-layered cortical plate and axon tract patterning are key features of cerebral cortex development. Abnormalities of these processes may be the underlying cause for many functional disabilities seen in disorders of cortical development. To generate recessive mouse mutants with phenotypes similar to human cortical malformations, N-ethyl-N-nitrosourea (ENU) mutagenesis was performed using two strategies. First, we used transgenic reporter lines to detect cortical patterning defects. We utilized mice expressing lacZ reporter genes in layer 2/3 and 5 of the cortex (R26- lacZ) or in a subset of cortical axons (TAGT1-tau-lacZ). Second, we employed a high-throughput RNA in situ hybridization of layer-specific markers as a primary screening tool. At day 5, lines were screened and we have established 15 lines with neurodevelopmental phenotypes. Five lines with disorganized cortical lamination have been identified, several of which displayed resemblance to lissencephaly or cobblestone heterotopia phenotypes. In addition, three lines showed abnormal cortical axon development. A microcephaly mutant and several neural tube closure mutants were also identified during the screening. Eight mutations have been mapped using whole genome single nucleotide polymorphism (SNP) genotyping, and one of the lamination mutants was found to have a mutation in reelin. To more rapidly identify the causal locus in other lines, we are employing whole genome sequencing strategies as a primary analysis. Mutants obtained from this mutagenesis experiment will facilitate the mechanistic analysis of cortical development and related human disorders. These findings prove the utility of ENU mutagenesis to recover alleles with roles in cortical patterning. Furthermore, this experiment demonstrated that the application of high-throughput, automated RNA in situ hybridization for large-scale screening is feasible.

Deciphering the complex genetic interactions contributing to learning and memory phenotypes in Down syndrome mouse models. Yann Herault, Arianna Franca, Arnaud Duchon, Damien Marschal, Clara Chevallerie, Patricia Lopez, Ignasi Absanosa, Victor Tybulewicz, Elizabeth Fisher, Sara Dienssens, Sabrina Luijser, Jean-Charles Bizot, Stylianos Antonarakis, Veronique Brault. 1) Translational Medicine and Neurogenetics, IGBMC, CNRS, INSERM, UdS, Illkirch, France; 2) Institut Clinique de la Souris,CNRS, INSERM, UdS, Illkirch, France; 3) Transgenese et Archivage Animaux Modèles, TAAAM, CNRS, UPS44, Orléans, France; 4) Genes and Disease Program, Center for Genomic Medicine, UAB, USA; 5) Centre d’Etudes Genétiques de l’Institut Curie, CEGIC, Curie Institute, Paris, France; 6) Centre National de la Recherche Scientifique, Unité Mixte de Recherche 3522, Paris, France.

The Down syndrome (DS) mouse model is a valuable tool for the study of the effects of trisomy 21 (Hsa21) and results in congenital heart defects in approximately 50% of DS newborns. The Mus musculus Ts65Dn model, with triplication of approximately 132 genetic orthologs to Hsa21, has various phenotypes corresponding to those found in human DS, including neonatal lethality and cardiovascular defects. We expect that dosage imbalance in Ts65Dn embryos causes the misexpression of triplicated genes, thereby disrupting expression of other genes throughout the genome, producing the observed cardiovascular abnormalities. We utilized proteome analysis to examine the array of proteins expressed at embryonic day E14.5, during the final stages of cardiac septation. Analysis of two-dimensional (2D) protein gels with Delta 2D software followed by tandem mass spectrometry (MS/MS) with Mascot data analysis allowed identification of proteins that are misregulated in the trisomic vs. euploid sibling embryos. Modification of methodologies was critical as very small masses of embryonic heart tissue were the starting material for protein extraction and analysis, and entailed minimizing volumes, eliminating steps, and increasing staining sensitivity. This work allowed identification of nine protein spots, such as cardiac myosin (MLC2a), reticulocalbin, and e-zinc, which showed significantly higher levels of protein in trisomic hearts than in euploid siblings. Analysis of protein spots significantly underexpressed in E14.5 trisomic hearts identified myosin regulatory light chain 2 ventricular isoform (MLC2v). As a critical component of chamber specification MLC2v overexpression could result in abnormal septation; compensatory action by MLC2a may mitigate this effect. This work represents a novel approach to evaluating the effects of dosage imbalance during development and has identified multiple proteins whose abnormal expression levels may disrupt the normal protein milieu during cardiogenesis to produce the pathological conditions seen in trisomic embryos with cardiovascular defects.
insulator function and paternal repression. Surprisingly, maternal insulator function is only disrupted in 149B imprinting. We have generated mice carrying microdeletions of 0.8kb (regulatory sites and disrupt the spacing of CTCF sites and CpGs at the ICR in a manner similar to those found in BWS patients. We find that while the 0.8kb deleted region is

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

POSTER: Mouse models of human disease

Syndrome (SRS) and several types of cancer. The purpose of our study is to characterize genetic and epigenetic mechanisms that regulate imprinted expression by examining (LOI) caused by deletions, duplications or epimutations are associated with a number of human diseases including Beckwith-Wiedemann Syndrome (BWS), Silver-Russell Syndrome (SRS) and several types of cancer. The purpose of our study is to characterize genetic and epigenetic mechanisms that regulate imprinted expression by examining H19 and IGF2, two imprinted genes that are co-regulated by an Imprinting Control Region (ICR) located between the two genes. At the maternal H19/IGF2 ICR, CTCF binds to create an insulator, which separates the maternal IGF2 promoter from downstream endosomal and mesodermal enhancers. This results in silencing of IGF2 while H19 is expressed on the maternal allele. Disruption of insulator function is associated with BWS. At the paternal H19/IGF2 ICR, DNA methylation is required for H19 silencing and paternal IGF2 expression. Loss of methylation at the paternal ICR is associated with SRS. Here, we investigate the role of cis regulatory elements at the H19/IGF2 ICR that are necessary for imprinting. We have generated mice carrying microdeletions of 0.8kb (IJSV) or 1.3kb (A3) at the ICR. These deletions significantly reduce the size of the ICR, delete potential regulatory sites and disrupt the spacing of CTCF sites and CpGs at the ICR in a manner similar to those found in BWS patients. We find that while the 0.8kb deleted region is dispensable for maternal insulator function, it is required for complete repression of paternal H19. On the other hand, the 1.3kb deleted region is required for both maternal insulator function and paternal repression. Surprisingly, maternal insulator function is only disrupted in A2,3 mutant tissues of mesodermal origin revealing a novel relationship between ICR-mediated silencing and the downstream enhancers. Studies are ongoing to dissect the underlying mechanism of this spatially restricted disruption of insulatin and imprinting.

The Power of the Collaborative Cross mouse resource population for mapping QTL associated with host susceptibility to Klebsiella pneumoniae infection. Fuad Iqbal1, Karin Vered1, Binnar Yalcin1, Izhak Oke1, Caroline Durwan1, Richard Mott1. 1) Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel-Aviv, Ramat-Aviv, Israel; 2) Wellcome Trust Centre for Human Genetics, University of Oxford OX3 7BN, UK.

Klebsiella pneumoniae (Kp) is a pulmonary pathogen causing severe pneumonia often associated with sepsis. With the rise of antibiotic resistance in bacteria, there is a need for alternative control methods. Here, we initiated studies aimed at mapping and subsequently identifying the host susceptibility genes to Kp infection in a high genetically diverse mouse resource population, the Collaborative cross (CC). In total, 434 mice of 73 CC lines were challenged by intraperitoneally (IP) with 10^4 CFU of Klebsiella pneumonia strain K2 and variety of traits, including mean survival time post infection, body weight at different time points during the challenge, were monitored for 15 days duration. Genomic DNA of the CC lines was genotyped with 620K single nucleotide polymorphic of mouse diversity array, and subsequently a QTL mapping was conducted using HAPPY software.

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wild-derived allele at this QTL significantly decreased white fat pad weight, body weight and serum levels of glucose and triglyceride in mice fed the low-fat diet. It was also reported that the high-fat diet decreased all of these outcomes for the transgenic mouse strains producing the wild-derived allele. However, no studies have been conducted to evaluate the effects of the substitution of the wild-derived allele in other genetic backgrounds.

151A

**Wild-derived Mus spretus strains: a resource for genetic resistance to Plague.** JEAN JAUBERT1,2, CHARLOTTE LEBLANC1,2, CHARLENE BLANCHET2, SYLVIE GARCI3, ELISABETH CARIEN4, CHRISTIAN DEMEURE5, ROBERT GEFFERTS2, CLAUDIA POMMEREKEN5, KLAUS SCHUGHART1, JEAN-JACQUES PANTHIER3, XAVIER MONTAGUTELL12. 1) Mouse Functional Genomics, Institut Pasteur, PARIS, France; 2) CNRS URA 2378, F-75015 PARIS, France; 3) Lymphocyte Proliferation, Institut Pasteur, PARIS, France; 4) Yersinia, Institut Pasteur, PARIS, France; 5) Department of Infection Genetics, Helmholtz Centre for Infection Research & University of Veterinary Medicine Hannover, Braunschweig, Germany.

Plague is caused by the Gram-negative bacterium *Yersinia pestis*. Laboratory mice are susceptible to plague. We have recently described that wild-derived Mus spretus SEG/Pas mice were exceptionally resistant (90%) to the virulent CO92 wild-type strain of *Y. pestis* in an experimental model of bubonic plague. We screened other *Mus spretus* derived strains and identified the STF/Pas strain as susceptible. QTL mapping in an intercross between SEG resistant and STF susceptible strains led to the identification of two genomic intervals on Chromosome 8. These QTLs are distinct from the three previously identified in a cross between SEG and C57BL/6J strains (Blanchet et al., 2011). Pre-congenic (NS) females carrying a heterozygous Chr5:SEG5T STF female littermates. Grazing experiments between STF and SEG showed that the SEG phenotype configuration was not transferred. This resulted in that: different mechanisms are at play between STF and B6 strains to result in the lethal outcome; 2) early innate immune response events are implicated in the resistance phenotype. Macrophages are well recognized as being at the forefront of innate immune response to *Y. pestis*. We extracted perilone macrophages from both parental *Mus spretus* strains and incubated them ex vivo for 3 hours with *Y. pestis*. Preliminary transcriptomic differences will be presented. Combination of QTL and transcriptomic data should help in unravelling some of the mechanisms involved in resistance to plague.

152B

**IRON DEFICIENCY UP-REGULATES DOPAMINE-RELATED GENES IN THE VENTRAL MIDBRAIN IN INBRED MICE.** Byron C. Jones1, Leslie C. Jellen1, Erica L. Unger1, Christopher J. Earley2, Lu Lu1, Robert W. Williams1, Xusheng Wang1. 1) Penn State University, University Park, PA; 2) The Johns Hopkins University, Baltimore, MD. The University of Tennessee Health Sciences Center, Memphis, TN.

Recently, we sought to identify genes that change in expression in response to low iron concentration in the substantia nigra, a feature of restless legs syndrome (RLS). We mapped quantitative trait loci for ventral midbrain (VMB) iron concentration in response to long-term feeding of either an iron-adequate or a low iron diet in mice. The mice were fed the Bxd recombinant inbred panel. We observed that brain iron loss differed markedly across the strains ranging from 0 to ~40%. Moreover, ventral midbrain (VMB) iron levels were correlated with dopamine-related phenotypes and pointed to a significant genetic variation on Chromosome 2. Within this QTL is the glial high-affinity glutamate transporter (*Slc1a2*), a putative candidate, as its expression was both correlated with VMB iron and cis-regulated. We then used microarray analysis to measure VMB iron gene expression differences in six strains showing differential susceptibility to iron loss. This revealed higher expression of *Slc1a2* in iron deficiency in susceptible strains, further supporting *Slc1a2* a candidate for modulating VMB iron. Additionally, microarray analysis highlighted iron deficiency-induced changes in several dopamine-related genes, including strain-specific down-regulation of *Drd2* and up-regulation of stratal cell-derived factor 1 (*Ccl2*) and heparin-binding beta-glycoprotein (Hbb-b1), key mediators of nigrostriatal dopamine functioning. The novel up-regulation of Ccl2 and Hbb-b1 is significant, as iron deficiency is hypothesized to contribute to RLS by its effects on the dopamine system, but the mechanisms are unknown. This is the first systematic analysis of differential susceptibility to iron deficiency in the brain and shows differences not only in iron loss but also in the transcriptional response to dietary restriction in the VMB. Supported in part by USPS Grant AG 21190.

153C

**Bmi1 is regulated downstream of EGRF by CK2 and PP1 in Astrocytomas.** Georgette N. Jones, Jessica J. Hawes, Isabella F. Newton, Robert G. Tuskan, Karlyne M. Reilly. Mouse Cancer Genetics Program, NIH/NCI-Frederick, MD. 

Bmi1 is a known chromatin-binding PcG oncogene, has been shown previously to play an important role in neural stem cell maintenance; however its regulation during tumorigenesis has not yet been elucidated. In the present study, we show that Bmi1 is over-expressed in mouse and human astrocytomas, and that the loss of the transcript resulted in decreased proliferation. Growth factor signaling studies revealed that EGRF signaling specifically led to rapidly increased phosphorylation of Bmi1, suggesting a potential mechanism by which microenvironmental signals can influence epigenetic change. More specifically, we show that regulatory post-translational modification of Bmi1 by Casein Kinase 2 (CK2) and Protein Phosphatase 1 (PP1) downstream of EGRF signaling are crucial for the oncogenic role Bmi1 plays in tumorigenesis in vivo. Taken together, these data demonstrate a novel mechanism whereby extra-cellular growth signals can lead to regulation of an oncogenic chromatin modifier.

154A

**Molecular Basis of the Kcnv2 Modifier Effect on Epilepsy.** Benjamin S. Jorge, Jennifer A. Kearney. Vanderbilt University, Nashville, TN.

The Scn2aQ54 transgenic mouse model has a progressive epilepsy phenotype due to a gain-of-function sodium channel mutation and exhibits strain-dependent variability in phenotype severity. We previously mapped modifier loci that influence Scn2aQ54 phenotype severity and identified Kcnv2, encoding the voltage-gated potassium channel subunit Kv8.2, as a modifier gene (Bergren et al., 2009, Mamm Genome). In another study we found that the susceptible SJL/J (SJL) strain had a 3-fold increase in hippocampal Kcnv2 expression compared to the resistant C57BL/6J (B6) strain, and double transgenic Kcnv2:Q54 mouse lines expressing higher levels of Kcnv2 had an increased incidence of seizures and accelerated mortality compared to lines expressing less Kcnv2 (Jorge et al., accepted 2011, PNAS). These data suggest that differences in Kcnv2 expression may underlie the observed strain dependent phenotype variability. The purpose of this study is to identify cis-regulatory elements that influence steady-state Kcnv2 expression. Sequencing of SSL genomic DNA 3kb upstream and downstream of the putative Kcnv2 coding region revealed a high degree of polymorphism between SSL and B6 strains. RACE experiments suggest multiple Kcnv2 transcription start sites and alternative splicing with strain-dependent variation in usage. We are currently performing RNA protection assays in order to confirm these results. Based on our present data, it appears likely that strain variation in cis-regulatory elements alters Kcnv2 expression. Future studies of promoter activity and mRNA stability assays will determine how these cis-regulatory elements affect steady-state gene expression.

155B

**CHRONIC MILD STRESS AND DRINKING IN THE DARK: A NEW MODEL TO STUDY THE EFFECTS OF GENES AND ENVIRONMENT ON ALCOHOL CONSUMPTION.** Sarita K. Kambhampati1, Byron C. Jones1, Sheila Sutti1, Sonia A. Cavigelli1, Christina Ragani1, Pierre Mormède2, Robert W. Williams1, Lu1. 1) Penn State University, University Park, PA; 2) Université de Toulouse, Toulouse, France; 3) University of Tennessee Health Sciences Center, Memphis, TN.

Chronic mild stress (CMS) is a procedure by which experimental animals are subjected to a variety of perturbations administered at unpredictable times. These include brief restraint in a box, loud noise, changes in light, dark cycle, exposure to intruder mice, exposure to incinerator smoke and predator exposure to intruder mice. In this experiment, we tested female mice from eight strains from the BXD/AIL panel. The main object of the study was to determine the effect of several weeks of CMS on alcohol consumption as an exploratory of a new model of stress-drinking. Our alcohol measure was drinking in the dark (DID). The protocol ran 9 weeks starting with 1 week of acclimation, one week (4 days) of DID, 6 weeks of CMS, one week of CMS/DID and one week of DID only. The DID protocol calls for removal of water and replacement with 20% (v/v) EtOH 3h after lights off. The length of alcohol availability was 2h for the CMS. For the DID experiment we had a CMS group and a normal husbandry group. At the conclusion of the experiment, all animals were reared for 15 min in centrifuge tubes and sacrificed for tissue analysis. In addition to EtOH consumption, we measured plasma corticosterone following restraint, corticosterone in feces, thymus weight, body weight (weekly) and gene expression in adrenals and hippocampus. The results showed the expected large, strain related variability in all measures. We also observed large variation among the strains in relevant measures in response to CMS and restraint. This is the first study of the effects of multiple genotypes in response to CMS in RI strains and shows promise for developing new models to relate chronic stress and alcohol drinking. Supported in part by USPS grants AA13499 and AA177509.
155C


Early life stress has often been implicated in the development of psychiatric disorders. Despite this, the factors that mediate the interaction between gene and environment remain largely unknown. Recent studies provide evidence that such effects are mediated by epigenetic processes.

Using a maternal separation paradigm, we investigated phenotypic and epigenetic changes following early life stress in two inbred strains of mice, C57BL/6j and DBA/2J. We found an increase in the corticosterone stress response in male, C57BL/6j mice that had undergone maternal separation compared to controls. Additionally, anxiety behaviour decreased in males but increased in DBA/2J females, DBA/2J males displayed reduced exploration, and baseline activity was altered in males of both strains following maternal separation.

Using a candidate gene approach we found altered levels of methylation in the hippocampus at several CpG sites across promoter regions of Nr3c1, Avp and Nr4a1 in maternally separated male mice. Following this we are investigating genome-wide methylation using Nimblegen Mouse DNA Methylation 385K Arrays, and genome-wide expression levels in the same tissue. We aim to discover novel differences in methylation profiles following maternal separation that correlate with expression changes.

We will employ a pathway analysis method to uncover any pathways that may be disrupted following maternal separation. Furthermore, the use of two inbred mouse strains allows us to investigate the genetic contribution towards both behavioral and epigenetic modifications.

157A

A genetic locus mapping to Chromosome 4 unrelated to collateral circulation determines cerebral infarction in a mouse model of ischemic stroke. Sehoon Keum, Douglas Marchuk. Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, NC.

In a mouse model of ischemic stroke, infarct volume is highly variable and strain dependent, but the natural genetic determinants responsible for this difference remain unknown. After permanent distal middle cerebral artery occlusion (MCAO), infarct volume was determined for 16 inbred mouse strains. Subsequently, we and others have demonstrated that the extent of pial collateral circulation in the brain is inversely correlated with infarct volume data for the inbred mouse strains. We also mapped the identical Chromosome 4 locus for infarct volume that was also mapped for pial collateral artery density in F2 (B6xBALBc) intercrosses. Intriguingly, we have found that one strain of mouse, C3H/HeJ (C3H), does not follow the correlation. Both B6 and C3H strains exhibit a similar large number of collateral vessels but the observed infarct volumes were significantly different between the strains. These two additional strains may provide evidence for the presence of additional loci controlling infarct volume, independent of collateral vessel number. To identify genetic determinants modulating infarct tissue damage, we performed quantitative trait locus (QTL) analysis of surgically-induced cerebral infarct volume. We have identified a genetic locus mapping to Chromosome 4 that modulates infarct volume, accounting for over 50% of the variation, with a LOD score of 5.3. Taken together, our new linkage data suggests that genetic variation underlying the Chromosome 4 locus might be associated with a different physiological phenotype from collateral circulation.

Identification of the genes underlying this new locus may uncover novel genetic and physiological pathways that modulate cerebral infarction and provide new targets for therapeutic intervention in ischemic stroke, and possibly other human vascular occlusive diseases.

158B

Possible Gene Silencing for a Subset of Targeted KOMP Alleles in Adult Mice. J. Kirov1, A. Cipollone1, B. Willis1, R. Araiza1, K. Lloyd2, D. West2, 1) Mouse Biology Program, University of California, Davis CA; 2) Childrens Hospital of Oakland Research Institute, Oakland CA.

A subset of targeting vectors used in the production of KOMP alleles (http://www.komp.org/) contain a LacZ (bacterial beta-galactosidase) reporter driven by the promoter of the targeted gene and a neomycin selectable marker driven by the human β-actin promoter. The insertion of heterologous DNA into mammalian genes can result in gene silencing and this has been particularly well described for viral repeat sequences. An analysis of ~80 heterozygous (Het) KOMP mutant mouse lines (one native wildtype allele, one targeted allele) revealed three instances wherein the LacZ reporter (see http://www.kompphenotype.org/) was not detected despite tissue expression surveys which predicted LacZ staining.

A probe for Actb was used as an internal reference. Snx27 is a sorting nexin gene family member, expressed highly in the CNS and moderately throughout other body tissues. Relative expression of the LacZ allele in brain and heart was 7% and 15% of the native Snx27 allele respectively. Similarly, the Kmo gene, a gene in the kynurenine metabolism pathway, is highly expressed in liver, spleen and kidney but LacZ staining was not detected in these tissues. In the Het Kmo mutants, we found that the LacZ allele mRNA was not detectable in kidney and expressed in the liver at only 10% of the level of the native Kmo allele. These findings suggest that the targeted allele has been silenced. However, further studies to sequence the targeted allele in order to rule-out frame shifts leading to nonsense-mediated decay, and/or to identify evidence of gene silencing (e.g., CpG island methylation, and/or histone modifications) are needed in order to confirm that silencing may mediate reduction in the abundance of the reporter allele transcript. Supported by NIH Grants: ZU1HG004080-04S1, 3U2R01RR024244-03S2.

159C

Voluntary ethanol consumption in Kcnj9 knockout mice. L. C. Kruse, A. M. Colville, L. B. Koerell, K. J. Buck. Behavioral Neuroscience, Oregon Health & Science University, Portland, OR.

Using behavioral models of physiological dependence and associated withdrawal, positional cloning, expression analyses, and a knockout model, we identified Kcnj9 as a high-quality quantitative trait gene (QTG) for ethanol, zolpidem and pentobarbital withdrawal in mice (J Neurosci 29:11662, 2009). Kcnj9 encodes GIRK3 (Kir3.3), a subunit member of a family of inwardly-rectifying K+ channels that primarily mediate postsynaptic inhibitory effects of Gi/o-coupled receptors. Ethanol withdrawal severity and consumption/preference are genetically correlated (negative) when tested independently (Mamm Genome 9:983, 1998). Further, loci on chromosome 1 that span Kcnj9 are implicated in addiction. To assess the role of GIRK3 in ethanol consumption, we compared GIRK3 knockout (KO) and wildtype (WT) littersmats (inbred C57BL/6J background) using a 2-bottle choice paradigm. Mice had continuous access to ethanol (3%, 6%, 10% and 20%, 4 days per concentration) and were then assessed for water, saccharin or quinine consumption. Multiple studies detect quantitative trait loci on human 1q associated with the diagnosis of alcoholism (Addiction Biol 15:185, 2010). Although these human QTLs are generally still suggestive and mapped to large regions, we have fine-mapped the mouse QTL to an interval syntenic to 1q23.2. Further, KO studies implicate GIRK3 in ethanol withdrawal and consumption, cocaine self-administration (Neuropsychopharmac 28:932, 2003) and morphine response (Pharmac Genomics 18:231, 2008). Given the otherwise normal behavior of GIRK3 KO mice, this suggests the possibility that GIRK3-containing channels may make a selective contribution to the effects of drugs of abuse. Future work will be needed to assess the role of this translational target in human studies, and the mechanism(s) by which GIRK3 affects alcohol/drug responses." AA011114, DA005228, AA10760, AA07468 and the VA."
POSTER: Mouse models of human disease

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

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Primary ciliary dyskinesia (PCD) is a pediatric syndrome caused by defects in motile cilia and flagella. Mouse models of this disease are critical tools for understanding the genetic causes of the disease and for testing new treatments. We have developed genetic mouse models that exhibit human mutations in genes critical for cilia function and flagellar motility. Using Cre-mediated recombination, we generated transgenic mouse lines for all known human ciliopathy genes. Mouse models now exist for all ciliopathies, including primary ciliary dyskinesia (PCD), Kartagener syndrome, situs inversus, and others. These models provide a powerful system to study the molecular mechanisms underlying ciliary function and disease.

16Q3

Modes of Action of miR86 in Developing Inner Hair Cells. Morag A. Lewis1, Jennifer M. Hilton1, Jing Chen1, Cordelia Langford1, Stijn van Dongen2, Cei Abreu-Goodger2, Matias Pipari3, Anton Enright2, Karen P. Steel1. 1) Wellcome Trust Sanger Institute, Hinxton, UK; 2) European Bioinformatics Institute, Hinxton, UK.

MicroRNAs are small non-coding RNAs which regulate target genes by binding to specific sites in their 3’UTR and preventing translation or causing degradation of the transcript. They show a wide range of spatial and temporal expression patterns and are capable of regulating multiple genes, playing important roles in the development of many different systems. However, most microRNAs and their target genes have yet to be investigated in depth. We have previously described diminuno, a mouse carrying an ENU-induced mutation of miR86 in the seed region that is crucial for correct target recognition. Diminuno homoygotes (Domdo/Domdo) have no cocklear response and extensive hair cell loss, while heterozygotes show rapid progressive hearing loss and hair cell anomalies. Microarrays carried out on organ of Corti RNA identified 96 transcripts with significantly altered expression in mutant animals. The wildtype seed region was enriched in the upregulated genes and the mutant seed region in the downregulated genes, meaning the mutation causes both loss of normal targets and the gain of novel ones. However, many genes with altered expression had no wildtype nor mutant target sequence and are presumed to be indirect targets. Among these are Slc26a5 (prestin), Gfi1, and Piprg, which were downregulated in hair cells; these are known to cause hair cell degeneration when absent and could partially or entirely explain the Dmdo phenotype. However, the network of genes connecting miR86 to its targets has yet to be described. We have identified potential candidates using multiple bioinformatic methods and investigated their expression in wildtype and homozygote mice. Understanding the links between the master regulator MIR96 and its ultimate targets will not only add to our knowledge of microRNAs but will also identify new genes involved in the highly specialised development of the inner ear and therefore suggest therapeutic targets to combat progressive deafness.

16Q4

Production of optimum humanized mouse model for familial amyloidotic polyneuropathy. Zhenghua Li, Kiri Araki, Ken-ichi Yamamura. Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Kumamoto, Japan.

An autosomal dominant disease, Familial amyloidotic polyneuropathy, is caused by the mutation of transthyretin (TTR) gene. Although we have generated transgenic mice that carry various mutant TTR genes, formation of mouse/human TTR heterotrimers has been shown to be inhibitory to dissociation and subsequent amyloid formation. To avoid the effect of mouse TTR, we have generated fully humanized TTR knockout mice using the transgenic mouse targeting method. Using this method, we showed that a human TTR cDNA with the PGK-puro cassette can be efficiently inserted into the mouse Ttr locus by Cre-mediated recombination, and that the human TTR cDNA was expressed in a tissue-specific manner under the control of the mouse endogenous Ttr promoter. To produce such a humanized mouse, it is critical to obtain a correct expression of a human cDNA after insertion into an mouse endogenous gene locus. Thus, we further examined the usefulness of PGK-puro cassette or IRES for the correct expression of human TTR cDNA. We generated four mouse lines, PGK(+)/IRES(+), PGK(+)/IRES(-), PGA(-)/IRES(+) and PGK(-)/IRES(-), using Cre and Flp-mediated recombination. mRNA levels of human TTR were examined by Northern blot and RT-PCR analyses. Protein levels of human TTR in liver and serum were examined by Western blot analysis. The mouse line containing the PGK-puro cassette, but not IRES, exhibited quantitatively and temporally similar expression of human TTR cDNA. Removal of the PGK-puro cassette significantly downregulated the expression of the cDNA. The insertion of IRES sequence upstream of the human TTR cDNA resulted in decreased transcription, even in the presence of the PGK-puro cassette. The mouse line containing IRES, but not PGK-puro, showed the lowest level of expression. These results suggest that the PGK-puro cassette is necessary to enhance the expression of the coexistant human cDNA in the mouse Ttr locus.

16Q8

The role of MYC during the initiation and progression of pancreatic ductal adenocarcinoma. Wan-Chi Lin1, Qian Zhang2, Rene Opavsky3, Dominick DiMaio1, Dean Felsher2, Michael Hollingsworth1, Matthias Hebok1, Kay-Uwe Wagner1. 1) University of Nebraska Medical Center, Omaha, NE; 2) Stanford University School of Medicine, Stanford, CA; 3) University of California, San Francisco, CA.

Pancreatic cancer is one of the most lethal human malignancies with a 5-year survival rate of less than 5%. Effective early detection modalities are currently not available, and tumors are typically diagnosed at advanced stages. The majority of pancreatic adenocarcinomas (PDACs) during the progression of precursor lesions (PanINs) into PDACs, somatic mutations within tumor susceptibility loci accumulate, including KRAS and INK4A. In addition, overexpression of MYC has been reported in a significant subset of primary PDACs, while the importance of this transcription factor for the initiation, maintenance, and progression of PDAC is poorly defined. Our studies show that the overexpression of MYC is a very early event in pancreatic carcinogenesis in humans, and its expression is maintained in a subset of advanced PDACs. Similarly, increased MYC expression in a mouse model for pancreatic cancer progression indicated that MYC is required for the proliferation and survival of cancer cells in primary and metastatic lesions, we have developed a novel mouse model that allows a spatially and temporally controlled expression of this oncogene in a ligand-controlled manner in the pancreas. Transgenic mice expressing MYC in the pancreatic ductal epithelium develop cancer within 6 months. The majority of these tumors (about 75%) are ductal lesions, and about 25% are poorly differentiated cancers. Ablation of MYC in PanIN lesions as well as primary pancreatic cancers results in cell death and tumor regression. Collectively, the results of our studies suggest that upregulation of MYC is associated with early cancer progression, and the growth and survival of MYC-induced pancreatic cancer cells depend on the continuous expression of the transforming oncogene.

16Q5

TNNI3K modulates cardiac conduction in a mouse model of cardiac sodium channelopathy. Elisabeth M. Lodder1, Brendon P. Scicluna1, Annalisa Milano1, Michael W. T. Tanck2, Connie R. Bezzina1. 1) Heart Failure Research Center, Exp. Cardiology, AMC, University of Amsterdam, Netherlands; 2) Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Center, University of Amsterdam, Netherlands.

Mutations in cardiac ion channels lead to rhythm disorders associated with a high risk of sudden cardiac death. As for other Mendelian diseases, incomplete penetrance and variable clinical expression complicate the management of patients with these disorders. Expanding the genetic variability in F2 progeny of 129P2 and FVB/N mice with the Sca1786678D0+ mutation, we have previously mapped a quantitative trait locus (QTL) on Chromosome 3 that influences the variance in the electrocardiographic PR-interval, a measure of atrio-ventricular conduction. Since genetic variation underlying a QTL may affect the phenotype through effects on gene expression, we carried out an expression QTL study (eQTL), mapping genetic factors influencing the transcript levels in the left ventricle. We identified 16 eQTLs that map within the 1.5 LOD drop interval of the QTL on Chr 3, 7 of which were cis-eQTLs. Of these 7 only Tnni3k maps to the SNP displaying the highest LOD score found for the PR-interval QTL. Furthermore, these cis-eQTLs only the D3 insulin transcript correlates with the PR-interval (r=0.28, p=0.003). Tnni3k encodes for troponin 1 cardiac-3 interacting kinase, recently identified as a genetic modifier of cardiomyopathy progression in mice. In vitro overexpression of Tnni3k in mouse cardiomyocytes leads to a hypertrophic response. In human a CNV located within TNNI3K has been associated with hypertension. Furthermore, a genome-environment interaction has been found for a SNP in TNNI3K in relation to coronary heart disease. Our data provide novel evidence for a role of this gene in controlling the PR-interval, indicating a role for Tnni3k in atrial and/or atrio-ventricular conduction. This study was funded by the Netherlands Heart Foundation (NHS 2005T024) and the Inter-University Cardiology Institute of the Netherlands (ICIN 06401).

16A6


After tuberculosis (TB) infection mice of the I/St inbred strain exhibit shorter survival time, a more rapid body weight loss, higher mycobacterial loads in organs, and a more severe lung histopathology than mice of the C57BL/6 strain. A previously accomplished genome wide scan for the quantitative trait loci (QTLs) involved in the TB infection
We have established a panel of congenic recombinant mouse strains bearing differential small segments of the 17 chromosome transferred from the TB-susceptible I/St mice onto congenic backgrounds. The region of interest mapped in this study involves 6q16.3-q21.1-2.2 genomic region with multiple significantly different functional elements, e.g., antigen processing and presentation, apoptosis, transcription, endosomal biology. Identification of candidates is in progress; however, this is complicated by the fact that the accompanying cloning of genes provided many polymorphisms between I/St and C57BL/6 mice.

167B
DEMONSTRATING RESISTANCE-MITIGATING EFFECT OF ARTEMESIA ANNUA PHYTOCHEMICAL BLEND WITH IN-VITRO CULTURES OF PLASMODIUM FALCIPARUM AND IN-VIVO WITH PLASMODIUM BERGHIEI ANKA IN MICE. KANGTHEIHE T. LUCY, HASSANALI AHMED, SABAOMAR HASSAN.
ABSTRACT Resistance of Plasmodium falciparum to drugs such as Chloroquine and Sulfadoxine-pyrimethamine is a major problem in malaria control. Artemisinin derivatives, primarily in combination with other drugs, are increasingly being used to treat malaria, reducing the probability that parasites resistant to the components will emerge. Although stable resistance to artemisinin has yet to be reported from laboratory or field studies, its emergence would be disastrous because of the lack of alternative treatments. The project was designed to demonstrate resistance-mitigating effects of phytochemical blend of Artemisia annua relative to pure artemisinin against the malaria parasite Plasmodium falciparum and on rodent malaria parasite Plasmodium berghei ANKA. For the in vitro experiments selection was undertaken on two cultures of P. falciparum D6 (CQ-sensitive strain originally from Sierra Leone) and W2 (CQ-resistant strain from Indochina), by exposing them to A. annua phytochemical blend and the pure artemisinin over 50 cycles at doses initially required to give 50% mortality (IC50) of the parasites. Dose-response effects of the blend and the pure compound was determined after 20, 30, 40, and 50 exposure cycles and compared to see if significant difference in development were in causing mortality of the parasites. The in vivo experiments mice were inoculated with the Plasmodium berghei ANKA parasite and thereafter given the test drugs. After 4 days the mice were passaged and paraesthesia determined to calculate the effective doses ED50 and the ED90. The ED90 got was utilized to study resistance under drug pressure in the mice. The nucleotide sequences of the possible genetic modulators of Artemisinin and Artemisia annua blend resistance (mdr1, tctp, and ap6) of sensitive and resistant parasites will be compared.

168C
Mutations in Mcmr9 expose the sensitivity of germ cells to genome instability. YUNHAI LUO1, SUZANNE HARTFORD1, CHEN-HUA CHUANG2, CHRISTIAN ABRAITE2, TERESA SOUTHARD3, JOHN SCHIMMEL4. 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Biomedical Sciences and Center for Vertebrate Genomics, Cornell University, Ithaca, NY.

The germine is the group of cells which carries and passes genetic information from one generation to the next. Because of this important role, it is critical for germ cells to maintain their genomes well. Indeed, there is evidence showing that spontaneous mutation rates in germ cells are lower than those in somatic cells. However, the mechanism behind such protection is not fully understood. Here, we characterized a mutant mouse Mcmr9(G743), which affects genome maintenance in germ cells. Mcmr9 is a member of the MCM gene family. Members of the MCM family, McM2-7, constitute the replicative helicase complex. Studies using the Xenopus egg extract system suggested that MCM9 is required for DNA replication initiation. Mice homozgotes for the Mcmr9(G743) allele are viable. Nevertheless, considering the vulnerability of DNA to damages during replication, mutations in Mcmr9 might cause genome instability. In agreement with this expectation, Mcmr9(G743) mice displayed a high incidence of tumor formation. Metaphase spreads of mouse embryonic (MEFs) showed that Mcmr9(G743) mice exhibited meiotic arrest and a lack of spermatogonia in seminiferous tubules of the testes. Female mutants had less oocytes than wild-type. Immunohistochemistry analysis of newborn gonad revealed a factor that drives obesity in our monosomy 21 heterozygous mice.

169A
Genetic modifiers in mouse models of motor neuron disease. CATHLEEN LUTZ, MELISSA OSBORNE, DON PETER LIU, KIMBERLY HUEBSCH, LEAH RAE DONAHUE. Genetic Resource Science, The Jackson Laboratory, Bar Harbor, ME.

Spinal Muscular Atrophy (SMA) is a neuromuscular disease characterized by degeneration of motor neurons and results in progressive muscular wasting and weakness. The majority of patients with SMA have mutations in the survival motor neuron gene 1 (SMN1). The SMN2 gene, which is highly homologous SMN1 copy that is present in all the patients, is unable to prevent the disease because not enough full length stable protein is produced from the SMN2 locus. The copy number of SMN2 correlates with longer survival and inversely with disease severity. However, it has been shown that SMN2 copy number is not an absolute predictor of disease severity; environment and other genetic factors have been shown to play a role (Burnet et al 2009, Oprea et al 2008). The Jackson Laboratory has partnered with the SMA Foundation to characterize and distribute mouse models of SMA. We started investigating the role of genetic background in SMA mice at the Jackson Laboratory in response to reports that type I mice were not surviving as long as originally published. Our investigation of the lines revealed that the genetic backgrounds were not completely congenic on FVB/N. Our subsequent work focused primarily on a high-fat diet exhibited a highly statistically significant increase in both fat mass and body fat percentage compared to their littermate controls. Next, we carried out the histopathological analysis of different tissues of mice fed on a high-fat diet, and observed that the livers of the heterozygous mice showed severe fatty changes compared to mild to moderate fatty changes in the livers of their littermate controls. We also conducted the microarray analysis and the qPCR analysis on adipose tissues of heterozygous and wild-type mice fed on a high-fat diet to find genes that might explain the observed obesity in heterozygous monosomy 21 mice. We conclude that a high-fat diet might be an environmental factor that drives obesity in our monosomy 21 heterozygous mice.

170B
Segmental monosomy of a conserved interval on mouse chromosome 16 syntenic to human chromosome 21. Anna Migalska1, Louise Van Der Weyden1, Ozama Ismail1, Mark J. Arends2, David J. Adams1. 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) Department of Pathology, Addenbrookes Hospital, University of Cambridge, Cambridge, United Kingdom.

We have generated a mouse model carrying a deletion spanning 1.6 Mb of the mouse Chromosome 16, from the Lpli to Usp25 genes, syntenic to human region 21q11.2-21.1. This model has been developed to investigate the possible contribution of the deletion region to the development of clinical features diagnosed in patients with monosomy 21 syndrome. As the clinical phenotypes observed in patients with partial monosomies of chromosome 21 are very heterogeneous, and range from the presence of only mild to moderate mental retardation and lack of other dysmorphic or congenital malformations to the presence of the variety of severe clinical symptoms, such as mental retardation, microcephaly, epilepsy, craniofacial, skeletal, cardiac and/or renal abnormalities, we have monossomy 21 mice to a robust phenotypic screening in order to thoroughly determine whether clinical features diagnosed in patients can be observed in our monosomy 21 mice. Analysis of the data obtained from the phenotypic screening directed our attention to the results coming from the dual-energy X-ray absorptiometry (DEXA) analysis. In particular, we observed that our heterozygous monosomy 21 mice fed on a high-fat diet exhibited a highly statistically significant increase in both fat mass and body fat percentage compared to their littermate controls. Next, we carried out the epigenomic analysis of dendritic cells conferring increased susceptibility to allergic asthma. Lyudmila Mikhaylova, Lester Kobernik, Alexey Fedulov. Brigham and Women's Hospital, Boston, MA.

In a mouse model of maternal transmission of asthma risk (Hamada et al, 2003) neonates of asthmatic mothers are more susceptible to asthma than pups of asthmatic fathers or normal parents, recruiting epidemiologic observation in humans. With an intentionally low-dose allergen sensitization, offspring of normal mothers do not show asthma
phenotype, however, the genetically and environmentally identical pups of asomatic mothers develop allergic airway hyperresponsiveness and inflammation, as well as systemic features of allergy. Splenic dendritic cells (DC) from the neonates of asomatic mothers are uniquely capable of polarizing recipients’ immunity towards pro-allergic responses and confer increased allergy risk when isolated prior to allergen sensitization (Fedullo et al., 2010). This suggests that DC are functionally skewed from birth, therefore the aim of the study was to determine the causal changes in the epigenome and transcriptome of these cells. We have used whole genome gene expression analysis using Affymetrix and Illumina gene chip microarrays, and whole genome DNA methylation analysis using Switchgear platform. Allergen-naive DC from the neonates of normal and asomatic mothers were compared for differences in expression and methylation profiles. We have found no evidence of significant differences in gene expression between the two groups of naïve DC. However, DC isolated from neonates after allergen sensitization did show differentially expressed genes. Epigenomic analysis identified almost 8000 regions (mapping to 5440 transcripts) that were hypomethylated, and about 4400 (3547 transcripts) that were hypermethylated in asomatic susceptible pups compared to controls, partially overlapping with differentially expressed transcripts in the DC from sensitized neonates. These results suggest that increased asthma susceptibility in the offspring of asomatic mice is conferred through in utero epigenetic changes in DC which lead to changes in gene expression.

172A
A Novel ENU Induced Model of Obesity and Type 2 Diabetes. Lee Moir, Liz Bentley, Roger Cox. MRC Harwell, Harwell Oxford, Didcot, Oxfordshire, OX11 0RD, UK. Obesity has a serious impact on health and has a genetic basis requiring further research to understand the genes involved. An F1 male was identified with an obese phenotype (61.4g at 14 weeks of age) from a dominant ENU mutagenesis screen carried out at MRC, Harwell. Weighing of backcross offspring show that the obese phenotype is inherited with affected mice observed from 6 weeks of age, coinciding with an increase in fat and lean mass. When challenged by an IPGTT at 12 weeks of age affected mice are glucose intolerant and hyperinsulinaemic (Table 1). Table 1. Body composition at 11 weeks and T=0 plasma glucose and insulin concentrations at 12 weeks for unaffected (n=15) and affected (n=15) B6C57 males. Mean ± SEM. t-test * P<0.001.

<table>
<thead>
<tr>
<th>Body Wt (g)</th>
<th>Fat Mass (g)</th>
<th>Lean Mass (g)</th>
<th>Glucose T0 (mmol/l)</th>
<th>Insulin T0 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected</td>
<td>32.3±0.6</td>
<td>5.5±0.4</td>
<td>23.1±0.4</td>
<td>10.7±0.3</td>
</tr>
<tr>
<td>Affected</td>
<td>46.1±0.7</td>
<td>16.2±0.3*</td>
<td>25.7±0.4</td>
<td>17.8±0.7*</td>
</tr>
</tbody>
</table>

Genome scan analysis indicated linkage to Chr13 and further genotyping narrowed the candidate region to 93.07-97.42 Mb. Whole genome sequencing was carried out by the Genomics Services Group at the WITCh, Oxford. Alignment and initial analysis of the resulting sequence identified 2 high and 1 medium confidence SNP's. Validation confirmed the two high confidence SNPs as coding mutations. Future work involves full characterisation of the phenotype and establishing the mechanism of the causative mutation.

173B
Uncovering the Molecular Mechanisms of Congenital Heart Disease Utilizing Mouse Genetics. Ivan P. Moskovitz, Joshua D. Bosman, Andrew D. Hoffmann. The University of Chicago, Chicago, IL.

Empirical evidence supporting a genetic basis for the etiology of Congenital Heart Disease (CHD) is limited and few disease-causing mutations have been identified. To identify novel CHD genes, we performed a forward genetic screen for ENU-induced CHD-causing mutations in mice. A screening protocol using the fetal-to-neonatal circulatory transition unmasked structural heart defects characteristic of CHD. Cardiac pathology revealed heart defects in 47 of 321 mutagenized lines. All identified structural abnormalities were analogous to described forms of human CHD. We mapped the locus responsible for heritable atrial septal defects in 6 lines (avc1-6). Furthermore, the phenotypic recurrence and variance patterns across all lines was similar to human CHD recurrence patterns, suggesting that “sporadic” CHD may have major genetic component. Molecular characterization of mutant lines identified a novel paradigm for cardiac septation and the operation of atrial septal defects. We showed that avc1 is a hypomorphic mutation in intralagellate transport in heart phenotype (avc1-723), required for cilia number marked (Hh signals) and Hedgehog (Hh) signaling. Avc1 is required for atrial cardiogenesis specific for the atrial septum and the pulmonary artery in mice using Genetic Inducible Fate Mapping. In the cardiac inflow, Hh-receiving cardiac progenitors migrated through the dorsal mesocardium to generate the atrial septum. Loss of Hh signaling during atrial septal progenitor specification caused atrial septal defects. We showed that Hedgehog signaling is necessary and sufficient for atrial septal progenitor fate in second heart field cardiac progenitors. These observations demonstrated that atrial septation is directed by progenitor cell specification rather than by positional information in the atria. Shh expressed in pulmonary endoderm was the responsible Hh ligand. Therefore, respiratory endoderm induced cardiac septa. We speculate that signaling from respiratory endoderm to second heart field cardiac progenators may underlie early events in the evolution of cardiac septation.

174C
Genetic regulation of Neurexin1 expression: an integrative cross-species analysis of schizophrenia candidate genes. Khyobeni Mozhu1, Xusheng Wang1, Jingchun Chen1, Megan M. Mulligan1, Jesse Ingles1, Xiangning Chen2, Lu Lu1, Robert W. Williams1. 1) Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN; 2) Department of Psychiatry, Virginia Commonwealth University, Richmond, VA.

Neurexin1 (NRXN1) is a large presynaptic type 1 transmembrane protein that has complex and variable patterns of expression in the brain. Sequence variants in NRXN1 are associated with differences in cognition, and with schizophrenia and other psychiatric disorders, which itself often differs by as much as 6-fold among normal strains of mice and is under strong genetic control. Here we use genetic mapping, high coverage genome sequence, and expression QTL (eQTL) analysis to study the expression of this gene. We profiled a family of 72 inbred progeny of a cross between C57BL/6J and DBA/2J using exon arrays and massively parallel RNA sequencing (RNA-seq). Expression of NRXN1 exons has high genetic correlation (r>0.6) due to the segregation of a strong trans eQTL on chromosome (Chr) 8 and a cis eQTL on Chr 17. These two loci are also linked to murine psychiatric relevance to schizophrenia candidate gene with high neuronal expression (PBSD). Numerous synapse and cell signaling genes, known psychiatric and schizophrenia candidate genes were co-expressed with NRXN1. Cross-species expression and protein interaction networks identified glycogen synthase kinase 3 beta (GSK3B) as one of the most consistent and conserved covariates of neurexin. This co-expression implies genetic association and potential functional interactions. We tested NRXN1 and GSK3B for epistatic interactions using the Molecular Genetics of Schizophrenia data set and found that variants in these genes jointly modulate risk.

175A
Large-scale SAGE (Serial analysis of gene expression) analysis by NGS (next generation sequencer) for quick molecular genotyping. Takuya Murata, Yuichi Ishitsuka, Ryutaro Fukumura, Shigeru Makino, Yui Nakai, Hayato Kotaki, Yoichi Gondo. Mutagenesis and Genomics Team, RIKEN BioResource Center, Tsukuba, Ibaraki, Japan.

It is a critical issue to quickly detect any traits of mutant mice derived from a gene-driven mutagenesis prior to months of various phenotyping of live mice. One potent assessment would be molecular phenotyping based on comprehensive transcriptome analysis. The SAGE combined with NGS (large-scale SAGE) should be quick and thorough enough to conduct such comprehensive transcriptome comparison between the mutant and control samples from any tissue of any stage. SAGE also fits well with NGS, since one short sequence (SAGE tag) represents one mRNA molecule; namely, huge numbers of NGS reads directly address digital gene expression (DGE) data themselves. Toward a standard approach by large-scale SAGE, we used our inbreed model mice with one amino-acid-substitution (C429S) mutation of the β-catenin gene (Best Poster Award in IMGC 2010), because the critical phenotypes of these mutant mice were just limited to the abnormal morphologies of internal genitalia, firstly appeared in E16.5. To find other molecular phenotypes, we compared the fetal brain transcriptomes. Both fetal virilization of brain and differentiation of internal genitalia are tightly associated with androgen, thyroid and GSK3B for epistatic interactions using the Molecular Genetics of Schizophrenia data set and found that variants in these genes jointly modulate risk.

176B
The Collaborative Cross mouse population for dissecting host susceptibility to mixed infection inducing alveolar bone loss. Asyar Naseh1, Yaser Salaymeh2, Ariel Shusterman1, Richard Mot1, Caroline Darran3, Ervin Weiss1, Yael Houri-Haddad1, Fund Iraqi1. 1) Department of Prosthodontics, Hadassah Medical Center, Israel; 2) Department of Clinical Microbiology and Immunology, Tel Aviv University, Israel; 3) Wellcome Trust Human Genome Centre, Oxford University, Oxford, UK.

Periodontal infection (Periodontitis) is a chronic inflammatory disease, which results in the breakdown of the supporting tissues of the teeth. Here, we have imitated a study to determine the phenotypic response of Collaborative Cross (CC) mouse population, to periodontal bacterial challenge using an experimental periodontitis model, and subsequently perform genome-wide search for quantitative trait loci (QTL) associated with host susceptibility to the diseases. On average, about 10 mice of each of different 50 CC lines (5 infected and 5 controls) were tested. Briefly, infected mice were orally challenged with Porphyromonas gingivalis and Fusobacterium nucleatum. The infection was repeated...
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three times at 2 day intervals. 42 days following the final infection, the maxillary jaws were harvested and alveolar bone volume was quantified using microCT. Currently, we have completed the analysis of 23 different lines, which showed variation in response to the co-infection. Six lines out of the tested 40 lines of control population were susceptible (P<0.05), while the remaining lines were resistant to alveolar bone loss. Basic bone volume of CC lines was significantly difference (P<0.05). Sex effect of the mice on bone volume of control and infected mice was tested across the different lines, and found not to be significant with P=0.54 and P=0.08, respectively. Both male and female were susceptible to H1N1 virus. By analyzing of 54 BxD mouse strains and using survival, time to death and bodyweight loss as traits, we identified QTL peaks on Chromosomes 5, 16 and 19.

178A Identification of Novel Modifier Loci Affecting Polypyosis in the BXII Recombinant Inbred Strains. Stephanie C. Nandi1, Rayneisha Watson1, Julie Innocent2, Beatriz Dulanto1, Arthur M. Buchberg1, Linda D. Siracusa1. 1) Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, 19107; 2) Hahnemann University Hospital, 230 North Broad Street, Philadelphia, PA, USA 19102.

Mutations in the Adenomatous Polyposis Coli (APC) gene are an early event in colon cancer. Individuals affected by the autosomal dominant disorder Familial Adenomatous Polyposis (APC) develop hundreds to thousands of polyps in the colon and rectum. APC mutations cause loss of function of the tumor suppressor APC protein, which functions to negatively regulate Wnt/β-catenin signaling. APC is a powerful model to study biological mechanisms that affect initiation, growth and progression of small intestine and colon tumors. Genetic background influences polyp number, size, and location along the length of the intestinal tract. These mice are powerful models to study biological mechanisms that affect initiation, growth and progression of small intestine and colon tumors. Genetic background influences polypl number, size, and location in Apcre1 mice. We showed that the Pla2g2a and Aspa1 genes are responsible for the protective Modifier of Min1 and 2 (Mom1 and Mom2) phenotypes. Hybrid progeny from a cross involving C57/HeJ (C57) females carrying a susceptible Mom1 locus and C57BL/6J (B6) Apc−/− male mice that were heterozygous in polypl number were crossed to parent or combination of protective alleles within the C3H genome. We utilized the BXII recombinant inbred series to uncover modifier loci that suppress polypl formation, even in the presence of susceptible Mom1 alleles. Several candidate genes were selected and tested for differential gene expression within resistant and susceptible strains. We describe the strategies used to identify the genes responsible for these protective effects, leading to a better understanding of the molecular and biochemical pathways involved in intestinal homeostasis and disease. Research supported in part by NCI grants to LDS and AMB. SCN8A is the recipient of a Professor Fredric Reiders Ph.D. Scholarship and a Ruth L. Kirkstein National Research Service Award.

179B A novel mutation and alternative splicing of sodium channel Scn8a (Na1.6). Janelle E. O'Brien1, Julie Jones1, Louise Dionne2, Miriam Meisler1. 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI, 48109; 2) The Jackson Laboratory, Bar Harbor, ME, 04609.

SCN8A encodes Na1.6, a neuronal voltage-gated sodium channel that is concentrated at the axon initial segment and nodes of Ranvier. Na1.6 is an important determinant of neuronal firing rate. Mice with mutations of Na1.6 exhibit motor and behavioral abnormalities including tremor, dystonia, and premature lethality (reviewed in Meisler et al., Genetica, 2004). We are studying the novel spontaneous mutant Scn8a-181B. Homozygous mutant mouse display tremor, progressive dystonia, and a reduced lifespan as well as greatly reduced cerebellar neuron firing rates. Mutant mice have a deletion of a single evolutionary conserved residue in the final transmembrane segment of the sodium channel. We are investigating a possible effect of this mutation on subcellular localization. The SCN8A gene contains two mutually exclusive exons, 18A and 18N, that are regulated in a tissue-specific and developmental pattern. Inclusion of exon 18A occurs only in mature neurons and encodes the full-length functional ion channel. Exon 18N encodes a truncated protein. Na1.6-18N is expressed in non-neuronal tissues and in fetal brain. We are using minigene constructs to investigate the molecular mechanism of this alternative splicing event. The role of neuron-specific splice factors in regulation of SCN8A expression will be described.

180C Bone density phenotypes in mice aneuploid for the Down syndrome critical region. Lisa E. Olson1,2, Saburrahman Mohan3,4. 1) Department of Biology, University of Redlands, California; 2) Ortho-Skeletal and Stem Cell Center, JL Pettus VA Medical Center, Loma Linda, CA; 3) Loma Linda University, Loma Linda, CA, USA.

Down syndrome (Trisomy 21) is associated with reduced bone density in humans, but it is unclear whether this is due to specific effects of chromosome 21 genes or lifestyle factors. Mouse models with aneuploidy of segments of mouse Chromosome 16 that are homologous to human chromosome 21 can be used to elucidate the mechanism by which Down syndrome phenotypes arise. Ts1Rhr and Ms1Rhr mice are trisomic and monosomic, respectively, for the hypothesized “Down syndrome critical region” containing several known Down syndrome candidate genes. Ts1Rhr mice, in contrast, showed decreases in weight, bone mineral density, and bone area from weaning to adulthood. Regional bone density was also decreased in the femur, tibia, and lower lumbar spine. The microarchitecture of three week old Ms1Rhr femurs was then analyzed using μCT. Volumetric density, total tissue volume, bone volume, and bone fraction were all reduced in both cortical and trabecular bone. Ms1Rhr trabeculae were thinner and had decreased connectivity. A 31.5% reduction in the level of insulin-like growth factor 1 in the serum was found, and we hypothesize that this is responsible for the bone density phenotype. We discuss bone-related genes in the region and propose that humans with distal chromosomal 21 deletions may exhibit reduced bone density.

181A Genetic refinement of the Mom5 modifier of intestinal tumorigenesis and characterization of the candidate gene, Rint-1. Karla L. Ottopher, Alicia Cleveland, Kimberly Bynoté, Karen A. Gould. University of Nebraska Medical Center, Omaha, NE.

Hereditary colorectal cancer syndromes display variable expressivity that results from unlinked modifier genes. These modifiers affect tumor latency, incidence, and multiplicity and are quantitative measures of tumor susceptibility. However, mapping these modifiers in human populations is extremely difficult. By contrast, aposcA103 mice, which develop intestinal tumors due to a germline mutation, provide a model to easily identify such loci. In our studies, aposcA103 mice on a B6 background develop ~40 intestinal tumors. However, when placed on other backgrounds, the Min mutation results in reduced mean tumor number as a result of modifier alleles. Using linkage analysis, we mapped one such modifier, Mom5, from the 129P2 strain to a 16.5 Mbp region on proximal mouse chromosome 5. This interval contains ~160 annotated and hypothetical genes. Ongoing studies to further refine the Mom5 interval involve the generation of B6.129P2-Mom5 recombinant congenic strains. We are also utilizing in silico approaches to identify potential candidate genes within the Mom5 interval. These analyses involve the identification of polymorphisms between the 129P2 and B6 strains from publicly available mouse sequence data and analysis of the potential impact of these polymorphisms using various bioinformatics tools. To date, these approaches have identified 129 non-synonymous variants in 44 genes within the Mom5 interval. In one of these genes, Rint-1, the B6 strain contains a SNP that impacts a critical residue in the N-terminal coiled-coil domain. This variant, which has been confirmed by targeted resequencing, results in the substitution of a threonine for a highly conserved isoleucine and is predicted to have a detrimental impact on protein functionality. Therefore, Rint-1 represents a good candidate for the Mom5 locus; however, other variants cannot be excluded. Ongoing studies will reveal what effect the Rint-1 variant has on protein expression, structure, and function, while the remaining variants will be evaluated via a bioinformatics pipeline.

182B Fine-mapping alleles for body weight in LGJ × SMJ F2 and F2 advanced intercross lines. Clarissa C. Parker1, Riyan Cheng1, Greta Sokoloff2, Jackie Lim2, Andrew D. Skol1, Mark A. Abney2, Abraham A. Palmer3,4. 1) Department of Human Genetics, University of Chicago, Chicago, IL; 2) Departments of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, NC; 3) Department of Medicine for Genetic Medicine, the University of Chicago, IL; 4) Departments of Psychiatry and

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Behavioral Neuroscience, the University of Chicago.

Body weight is an important determinant of health and fitness in both humans and agricultural animals, yet the underlying genetic mechanisms are poorly understood. We measured variation in body weight using a combined analysis in an F1 intercross and an F2 advanced intercross line (AIL). These lines were derived from inbred LG/J and SM/J mice, which were selected for large and small body size prior to inbreeding. Body weight was measured at 62 ± 5 days of age. Using an integrated GWAS and a novel forward model selection approach, we identified 11 significant QTL that affected body weight on 10 different chromosomes. With these results we developed a full model that explained over 18% of the phenotypic variance. The most significant 5-LD support interval was 5.55 Mb, which is a significant improvement over most existing body weight QTL mapping analyses. We identified coding SNPs between LGJ and SMJ mice which further narrowed our list of candidate genes. The gene Sox5 is located in one of the three smallest QTL regions and contains multiple polymorphisms between the LGJ and SMJ strains that may influence mRNA stability. A knockout of Sox5 has already been reported to have lower body weight and Sox5 is biologically related to Sox6, which has been associated with obesity in human GWAS; these observations strongly suggest that Sox5 underlies one of our QTLs. Our results demonstrate that a relatively small number of loci contribute significantly to the phenotypic variance in body weight, which stands in marked contrast to the situation in humans. This difference is likely to be the result of strong selective pressure and the simplified genetic architecture, both of which are important advantages of our system.

183C

LacZ Reporter Gene Expression in 81 KOMP Heterozygous Mutants: Sensitivity, Staining Patterns and Functional Inferences. Ravi K. Pasumarthi1, Brian Bardoin1, Andrew Mamalis1, Esi Djan1, Amanda Trainer1, Stephen M. Griffin2, K. C. Lloyd2, 1) Mouse Biology Program, School of Veterinary Medicine, University of California, Davis, CA; 2) Childrens Hospital of Oakland Research Institute (CHORI), Oakland, CA.

The vector used for “knocking out” targeted genes as part of the Knockout Mouse Project (KOMP) contains a LacZ reporter driven by the endogenous promoter of the targeted gene. Therefore, in mice carrying the KOMP allele, the tissue expression patterns of the gene can be ascertained by histochemical staining for LacZ. As a component of the KOMP-312 Phenotyping Pilot Project, we are completing both whole-mount (WM; 1M & 1F) and frozen section (FS; 1M & 1F) LacZ staining in heterozygous mutants. One wild-type control is stained for every 7 mutants to control for nonspecific LacZ staining. To date, we have evaluated LacZ staining in 81 mutants and annotated images are available on the project webpage (www.kompphenotype.org ). For WM and FS staining, we scored staining in ~50 and 42 tissues respectively. Of the 81 mutants we have evaluated to date: 17 show specific staining in only one organ/tissue; 27 have specific staining in 6 or more organs/tissues; and 21% have no specific LacZ staining.

Interestingly, 46% of mutants show LacZ staining in brain. In ~20% of the mutants with LacZ staining, we find unique expression patterns not previously reported in the literature, suggesting new and unknown gene function. For example, LacZ staining for Ninj1, a nerve-injury induced protein, is widely observed in cardiovascular smooth muscle and connective tissue, suggesting an important function of this gene family in the cardiovascular system. More than 40% of the mutants we have evaluated to date are stained in heart, suggesting that the LacZ reporter may be a useful screening tool for genes with hyperkeratosis phenotype in humans with presumed loss-of-gene-function mutation. This data collectively illustrate the value of LacZ reporter gene staining in knockout mutants to ascertain normal tissue expression patterns and provide functional annotation. Supported by NIH Grants: 3U01HG004080-04S1, 3U42RR022444-03S2.

184A

Genetic Heterogeneity in Commercial Outbred Mice from Taconic. Ana V. Perez2, Jan Gray2, Stephen Festin2, Gerald W. Both2. 1) Genetics Department, Taconic, Hudson, NY; 2) Research and Development, Taconic, Rensselaer, NY.

Natural populations are usually a mix of individuals that are genetically diverse. Outbred mouse stocks can be the optimal model for populations that are genetically diverse. Traditional outbred mouse stocks provide a resource of outbred mice to the scientific community, but data on population genetics of these outbred stocks are sparse. Taconic assesses yearly the genetic variability of its outbred stocks and to maintain their heterozygosity, Taconic breeds these with a rotational breeding method that minimizes inbreeding properly. Commercial mouse breeders provide a resource of outbred mice to the scientific community, but data on population genetics of these outbred stocks are sparse. Taconic assesses yearly the genetic variability of its outbred stocks and to maintain their heterozygosity, Taconic breeds these with a rotational breeding method that minimizes inbreeding properly. Traditionally genetic variability was tested through protein polymorphisms and through restriction fragment length polymorphisms (RFLPs). Recently, Taconic adopted the Illumina platform that tests SNPs to monitor the genetic background of its inbred stocks and outbred stocks. Using a commercially available 1449 SNP MBD panel, we have assessed the genetic variability of Taconic’s outbred stocks by calculating their Heterozygosity (H) and the number of polymorphisms. We tested the following stocks: ICR (IcTac:ICR), Swiss Webster (Tac:SW), Swiss nude (NTac:NIHS-Foss™), NMRI (BomTac:NMRI), NMRI nude (BomTac:NMRI-Foss™), which are 100% Swiss-derived, and of Black Swiss (NTac:NIHS), ICR scid (IcTac:ICR-Prkdc™), NCR nude (Ctac:NTac-Foss™), and NIH-III (Tac:NIHS-Lyn(Foss™)Bk/Btk™), which are partially original of Swiss. C57BL/6NTac and Swiss-derived inbred strains were used as comparison. From the 1449 SNPs, 1402 scoreable SNPs, 1133 were polymorphic among all Swiss-derived outbred stocks, 832 among five Swiss-derived inbred strains, and 1303 among all Swiss-derived mice tested. The heterozygosity of the outbred stocks varied from 0.148 in the main Swiss Webster population to 0.006 in Black Swiss mice. In summary, most of Taconic’s outbred stocks present reasonable genetic heterozygosity and in stocks where a particular allele has been purposely fixed such as for example the Foss™ mutation, the level of heterozygosity decreases when compared to the outbred stock without that particular allele.

185B

Milk ejection in mice LG/J × SMJ. Andrea Cristina Peripato, Carolina Purcell Goes, Bruno Sauce. Genetics and Evolution, Federal University of Sao Carlos, Sao Carlos, Brazil.

In mammals, milk is a primary contribution to offspring survivorship and growth in early stages of life. This source of nutrients must be provided to the offspring right after birth, making milk ejection an important maternal function. In this study we investigated milk ejection in the first day postpartum (D1), referred to as ME, in LG/J and SMJ inbred mice strains and their intercross. ME was measured by milk presence (MP) or absence (MA) in the pups’ stomach at D1. We found that LG/J females have poor ME when compared to SMJ females (39% vs. 83%, p<0.001), F1 and F2 generations (both showing about 95% of ME, p>0.001). Differences also found among SMJ females and F1 and F2 generations (p=0.01, p=0.01, respectively) suggest heterosis for ME. MA is inversely associated with viability of F3 offspring, with survival rate of 56% for MA vs. 98% for MP (p=0.001). Nonetheless, pups that survive this absence, mostly males, have a tendency to be heavier at weaning (~1.33 g; p<0.001) than those who have MP. This weight gain is not accompanied by growth of the animal (tail length differing ~0.13 cm; m.s.); rather, it appears to be due to higher fat deposition, according to necropsy data (~0.77 g of total fat, p<0.01) in males with MA. It seems like early life metabolic adaptations that promote animal survival to MA gives them a higher tendency to fat deposition; that is, a “Thrifty phenotype". We performed a QTL analysis using 101 markers distributed across all chromosomes and ME data in F2 animals. Our results reveal a single QTL at distal position on Chromosome 3, highly significant with a predominantly additive effect, which accounts for about 8% of the variation in ME. In the interchromosomal two-way genome-wide scan for epistatic loci we found a major network involving 11 loci in ten connections, which includes the single QTL, and five small ones, that together explain over 19% of ME in this intercross. Financial Support: FAPESP.

186C

GETWISE, a new method to study gene function in live mice. Christophe Poirier, Natalia Bogatcheva, Alexander Verin. Vascular Biology Center, Georgia Health Sciences University, Augusta, GA.

We are developing a new ES cells based method to study the function of any given mouse gene in vivo. This method is called GETWISE, for Genetically Targeted With Inducible Specific Expression. With this method, we will be able to generate a single mouse transgenic animal sufficient to study the effects of both loss of expression and over expression of the target gene in dose-dependent, time-dependent, and tissue-specific manners. GETWISE plasmids have been designed to target any type of genes. From these plasmids, targeting vectors can be generated by 1 or 2 rounds of molecular cloning. As a proof of concept to validate our method we have successfully targeted 8 genes. By comparing with conventional targeting methods, we found that our method of homologous recombination in ES was over 10 times more efficient. In GETWISE animals, transcription of the targeted gene is transferred from the endogenous promoter to a tetracycline inducible promoter. In addition, the endogenous promoter is driving the expression of the firefly luciferase reporter gene which allows robust monitoring of the targeted gene expression. The tissue-specific over-expression of the target gene can be achieved in crosses between GETWISE mice and transgenic mice expressing rtTA in a tissue-specific manner, or in more complex crosses with mice expressing CRE recombinase in tissue-specific manner and mice carrying a ubiquitously Rosa26LoxP-STOP-LoxPProrTA transgene. Time-specific over-expression of the gene will be triggered by adding doxycycline to the animal/animal mother water. In GETWISE animals, it will also be possible to achieve post-developmental silencing of the gene of interest. For that, GETWISE knockouts should be crossed with mice carrying a ubiquitous or tissue-specific CRE recombinase, or a ubiquitously expressed Cre recombinase under tetracycline control. The drinking water with the GETWISE method, it is possible to develop mice strains where level of expression for any gene can be controlled in any tissue at any time.

187A

Mouse models for the study of SP012 splicing isomers in miceois. Florence Pratto1, Mariana Bellani2, R. Daniel Camerini-Otero1. 1) Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD; 2) LMG, NIA, NIH, Baltimore, MD.

Meiotic recombination is initiated by the formation of programmed DNA double-strand breaks (DSB) catalyzed by the SP01 protein. This protein is widely conserved and

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Introduces DSB in a reaction in part similar to that described for type II DNA topoisomerases. Homologs of Spo11 have been isolated from mouse and human and it has been shown that there are many alternatively spliced variants of the protein and two major forms (α and β) are readily detected in testes. Analysis of isolated spermatocytes and juvenile mice has shown that the two isoforms have distinct transcription kinetics. Spo11 β transcripts (including all 13 exons) are found in early stages of prophase I and Spo11 α transcripts (exon 2 skipped) are mainly synthesized past pachytene stage. Since breaks are introduced in early prophase, the simplest model suggests that the larger form of Spo11 is the catalytic isoform that generates the DSBs, while the smaller form plays a structural role on synapsed chromosomes. In females, Spo11 β transcripts kinetics is similar to that of spermatocytes, but Spo11 α transcripts peak in early prophase (zygotene) and decline thereafter. The fact that both female and male meiocytes generate Spo11 alternative transcripts during prophase I, argues against a gender-specific function for Spo11 isoforms. Therefore, the study of mouse deafness mutants has lead to the identification of a number of human hearing impairment genes. The novel ENU-induced mouse deafness mutation nse5 is therefore a potential model of human hearing impairment. Homozygous nse5 mutants have abnormal auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) to sounds up to 80 decibels (dB) at frequencies ranging from 4 to 90 kilohertz (kHz), demonstrating a 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. Histological analysis of the inner ear reveals early death of the outer hair cells of the cochlea. Genetic analysis of 500 intercross progeny has localized the mutant locus to a 715 kilobase (kb) interval on mouse chromosome 15. A missense mutation at a highly-conserved amino acid was found in the mouse homologue of the yeast asparagine-linked glycosylation-10 gene (Alg10b), which is within the critical interval for the nse5 mutation. A 20 kb transgene containing a wildtype copy of the Alg10b gene rescues the phenotype nse5/nse5 homozygous animals. Targeted inactivation of the Alg10b gene is currently under investigation. The study provides the clearest example to date of the critical link between protein glycosylation and the normal development of the auditory apparatus and suggests a strong candidate gene for human genetic hearing impairment.

SPO11

关联交易的cilia proteins Cep290和Mkk4是通过结构和功能性相互影响的。Rivka A. Rachel1, Helen L. May-Simmer2, Shobi Veler3, Norimoto Gotoh1, Byung Youn Choi1, Jeremy McIntyre1, Jeffery R. Martens2, Thomas B. Friedman1, Hemant Khanna1, Matthew W. Kelley1, Anand Swaroop2. 1) N-NRL, NEI, NIH, Bethesda, MD; 2) NIDCD, NIH, Bethesda, MD; 3) Dept of Pharmacology, Univ of MI, Ann Arbor, MI; 4) Dept of Ophthalmology & Visual Sciences Univ of MI, Ann Arbor, MI.

Primary cilia act as biological sensors with the extracellular environment. Malfunctions in sensory cilia can lead to related and overlapping multisensory disorders referred to as ciliopathies. These conditions are characterized by syndromic phenotypes including variable deficits in brain development, renal function, and digit formation. Such patients often develop an early form of retinal degeneration and may also have hearing loss. Mutations in centrosome-cilia protein CEP290 are responsible for multiple inherited syndromic ciliopathies and are responsible for 20-25% of Leber congenital amaurosis (LCA), an early-onset retinal degeneration. The DSD (deleted in sensory dystrophy) domain of CEP290 is conserved among species and appears to be involved in the development of primary cilia. Genetic analysis of 500 intercross progeny has localized the mutant locus to a 715 kilobase (kb) interval on mouse chromosome 15. A missense mutation at a highly-conserved amino acid was found in the mouse homologue of the yeast asparagine-linked glycosylation-10 gene (Alg10b), which is within the critical interval for the nse5 mutation. A 20 kb transgene containing a wildtype copy of the Alg10b gene rescues the phenotype nse5/nse5 homozygous animals. Targeted inactivation of the Alg10b gene is currently under investigation. The study provides the clearest example to date of the critical link between protein glycosylation and the normal development of the auditory apparatus and suggests a strong candidate gene for human genetic hearing impairment.

A QTL for body fat (Adip5) is trapped in a congenic strain. Danielle R. Reed, Cailu Lin, Anna Lysenko, Amin Khoshnevisan, Alexander Bachmanov. Monell Chem Senses Ctr, Philadelphia, PA. Obesity is determined by the amount of lipid stored in adipocytes, which aggregate with other cell type to adipose depots, sometimes called fat pads. There are five large depots in the mouse: the gonadal, retroperitoneal, subcapsular, inguinal and mesenteric, as well as several smaller ones (e.g., pericardial). While overall fatness is highly heritable, the weight of individual depots have both overlapping and independent regulation. Our focus is on Adip5, a QTL identified from an intercross between C57BL6/ByJ and 129P3/J. Through a series of backcrosses and marker assisted selection, a consomic strain was created which served as a starting point for congenic construction.

Molecular characterization of the translocation breakpoint in the Down syndrome mouse model, Ts65Dn. Laura Reinholtz, Yueming Ding, Griff Gilbert, Lucy Rowe, Mary Barter, Doug Hinerfeld, Leah Rae Donohue, Cathleen Lutz, Muriel Davison. The Jackson Laboratory, Bar Harbor, ME.

Ts65Dn is a mouse model of Down syndrome; a human syndrome that results from Chromosome (Chr) 21 trisomy and includes a variety of congenital defects and post natal defects. Ts65Dn mice have segmental trisomy for distal Chr 16, a region sharing conserved synteny with human Chr 21. As a result, this strain harbors three copies of over half of the human Chr 21 orthologs. The trisomic segment of Chr 16 is present as a translocation chromosome (MMU 17^a), the originating breakpoints of which remain undefined at the molecular level. The breakpoint region on Chr 16 has been mapped to a relatively short interval (~3 Mb), while the Chr 17 breakpoint has been mapped to an interval of ~10 Mb. Therefore, while the gene dosage of Chr 16 alleles is well defined, the dosage of proximal Chr 17 alleles remains unknown. To molecularly define the Chr 16 and Chr 17 breakpoints, we used a selective enrichment and high-throughput paired-end sequencing approach. Two 1M feature arrays with overlapping probes (3 bp offset, ~60 bp probes) were used to capture Chr 16 sequence between Ncam2 and Apj, as well as Chr 17 sequence between D17Mit19 and D17Mit158, for a total of ~10 Mb of target sequence. This approach provided significant oversampling of the regions surrounding the breakpoints and by extension, significant enrichment for the most informative reads: paired end reads with mate pairs mapping to two different chromosomes, Chr 17 and Chr 16, and individual reads spanning the breakpoint. Combined mapping and de novo assembly of these data provide, for the first time, molecular characterization of the translocation breakpoints in this strain. Using these data, we now provide complete characterization of gene dosage in Ts65Dn mice, the basis for a robust genotyping assay and a straightforward, methodological approach by which high throughput sequencing can be used for the discovery of translocation breakpoints.

Non-trisomic boxhome gene and Sox9 expression alters craniofacial development in a Down syndrome mouse model. Randall J. Roper1, Cerie N. Billingsley1, Jared R. Allen1, Joshua D. Blazek1, Douglas Baumann2, Abby Newbauer1, Andrew Darrah1, Brad C. Long3, Brandon Young3, Mark Clement4, R. W. Doerge2. 1) Dept of Biology, Indiana Univ Purdue Univ, Indianapolis, IN; 2) Dept of Statistics, Purdue Univ, West Lafayette, IN; 3) Genomics Core, Scripps Florida, Jupiter, FL; 4) Dept of Computer Science, Brigham Young Univ, Provo, UT.

Trisomy 21 results in phenotypes including cognitive impairment, craniofacial dysmorphology, and heart defects collectively referred to as Down syndrome (DS). Although it is known that three copies of human chromosome 21 (Hsa21) causes DS, how trisomy alters developmental and genetic mechanisms to cause DS phenotypes is not well understood. Craniofacial alterations are a distinguishing feature of DS and may lead to complications in breathing, eating, and communication. Ts65Dn mice are trisomic for orthologs of about 188C A Defect in the N-Linked Glycosylation Pathway Causes Nonsyndromic Hearing Impairment in Mice. Frank J. Probst1, Rebecca R. Corrigan1, Daniela del Gaudio1, Simon S. Gao2, Andrew P. Salinger1, Hsiao-Yuan Tang2, Raye L. Alford3, John S. Oghalai4, Monica J. Justice5. 1) Molec & Human Gen, Baylor Coll Med, Houston, TX; 2) Bioengineering, Rice University, Houston, TX; 3) Molecular Carcinogenesis, MD Anderson Cancer Center, Houston, TX; 4) Otolaryngology - Head and Neck Surgery, Baylor College of Medicine, Houston, TX; 5) Otolaryngology - Head and Neck Surgery, Stanford University, Stanford, California.

Craniofacial alterations are a distinguishing feature of DS and may lead to complications in breathing, eating, and communication. Ts65Dn mice are trisomic for orthologs of about 189C Adip5 has been successfully trapped in a congenic strain and the original phenotype identified in the first intercross has been retained. Our goal is to reduce the size of the interval through additional backcrossing and to identify a short list of candidate genes. This new results for a potential synapsis function for SPO11 β isoform is responsible for introducing the breaks. New results for a potential synapsis function for Spo11 α will be discussed.
half of the genes found on Hsa21 and exhibit DS-like craniofacial alterations including a small mandible. Our prenatal analyses found Ts65Dn embryos at 13.5 days gestation (E13.5) display a small mandibular process as compared to normal, mandibular sized embryos, suggesting a relative instead of actual micrognathia originates during early development. Developing neurological and cardiac tissues were also altered in E13.5 trisomic embryos. In contrast to an expected increased expression of trisomic genes, only 155 non-trisomic genes were differentially expressed in the developing E13.5 mandible, including twenty genes containing a homeobox DNA binding domain. Additionally, non-trisomic Sox9, important in skeletal formation and cell proliferation, was upregulated in Ts65Dn mandibular precursors and overexpressed in trisomic Meckel’s and hyoid cartilages. Our results suggest trisomy causes disruption in downstream expression of non-trisomic genes early in development leading to structural changes associated with DS. The identification of genetic pathways disrupted by trisomy is an important step to propose therapies to ameliorate craniofacial abnormalities in DS and other congenital disorders.

193A Midline subcortical nodular heterotopias and partial callosal agenesis in a spontaneous mutation a recombinant inbred strain. GD Rosen1, N. Aoxaluay1, EG Griffin1, AJ Newbury1, L. Koganti1, D. Truong2, RH Fitiz2, Z. Li1, L. Lu1, X. Wang2, RW Williams3. 1) Neurology, BIDMC, Boston, MA; 2) Psychol, UCONN, Storrs, MA; 3) Anat & Neurobiol, UTHSC, Memphis, TN.

Disruptions of neuronal migration are associated with a wide variety of neurological disorders, including profound mental retardation, epilepsy, and developmental dyslexia. We recently discovered an extant strain of mice (BXD29-Tg(Tle4tmP2/1)1Ctgf) with a spontaneous mutation that causes bilateral nodular subcortical heterotopias and partial callosal agenesis. The wildtype (WT) BXD29/Ty was re-derived from a frozen 1979 stock (BXD29/Tyj).

The mutant phenotype is unlikely to be related to the previously reported Tbr4 mutation in this strain, as two other strains with this deletion had normal cortical morphology. Immunohistochemical staining for the subplate laminar marker Cgf revealed that the heterotopias are subcortical. BrdU birth dating studies and Cau+ (layer 2-4 laminar marker) immunoreactivity indicate that the majority of neurons in the heterotopia are generated relatively late in gestation. FosP2+ neurons, which are found exclusively in layers 5-6 in the WT, are located both in layers 5-6 and in upper cortical layers throughout the neocortex. There are also anomalies in FosP2 immunoreactivity in the thalamus and hippocampus. From a developmental perspective, there is evidence of incipient heterotopia formation at postnatal day (P) 1, and heterotopias are observed by P5.

We tested these mutant and WT animals on a gap detection task that has been previously shown to a sensitive predictor of neuronal migration disorders. The mutant strain was remarkably deficient, being unable to attenuate their startle response with gaps as large as 300 ms.

Breeding experiments confirm that this is an autosomal recessive mutation. We have sequenced (whole genome) the mutant strain (8X) using next generation short read technology (SOLID 4.0). We are also comparing whole brain RNA-seq data for WT and mutants.

194B Using Sleeping Beauty transposition to identify gene networks involved in a mouse model of mammary cancer. Jacquelyn J. Roth1, Richard C. Crist1, Jeffrey R. Horner1, Agnieszka Z. Wiatkiewicz1, Michael P. Lissanti1, Gregory E. Gonye1, Aaron Sarver1, Arthur M. Buchberg3. 1) Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA; 2) Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA; 3) Stem Cell Biology and Regenerative Medicine, Thomas Jefferson University, Philadelphia, PA; 4) Biostatistics and Bioinformatics, Masonic Cancer Center, University of Minnesota, Minneapolis, MN. Approximately 1 in 8 women and 1 in 1,000 men in the US will develop breast cancer during the course of his or her lifetime. Currently, only about 10% of breast cancers can be attributed to known genetic mutations. The purpose of this study is to develop a murine model of mammary tumorigenesis that systematically allows identification of oncogenes and tumor suppressor genes via sleeping beauty (SB) transposon (Tn) mobilization. SB is a mobile DNA element that has been shown to potentiate the development of tumors in mice by insertional mutagenesis. We are using the SB mouse model of tumorigenesis in combination with a mammary tissue specific promoter to create mammary tumors in wt and Ctgf-/- mice. We are interested in the role of the loss of Ctgf in mammary tumors because i) Ctgf is located in a region often associated with loss of heterozygosity (LOH) in Breast cancer and ii) a dominant negative mutation in Ctgf has been identified in 16% of Breast cancers. Thus far, we have isolated Tn insertion sequences from over 75 mice. We have isolated insertions that result in the loss of Ctgf in all 20 of the knockouts we have screened.

194C The deleted in liver cancer 1 gene (Dlc1) expression during mouse embryonic development. Mohammad G. Sabbir, Michael Mowat. 1) Manitoba Institute of Cell Biology, CancerCare Manitoba, 675 McDermot Avenue, Winnipeg, Manitoba R3E0V9, Canada; 2) Department of Biochemistry & Medical Genmetics, University of Manitoba, Winnipeg Manitoba, Canada.

Abstract: Introduction: The deleted in liver cancer 1 (Dlc1) tumour suppressor gene encodes a Rho GTPase activating protein that act as a negative regulator of RhoGTP. Although the cellular function of Dlc1 gene has been demonstrated in cell culture experiments, its physiological function is still need to be explored in vivo. The Dlc1 gene has at least 3 major full length transcriptional isoforms expressed under the influence of 3 alternative promoters in the mouse. We have previously established a mouse strain containing a gene trap (gt) insertion, which specifically reduces the expression of the major 6.1 kb transcriptional isoform (isoform 2) of Dlc1, thus creating a hypomorphic. The Dlc1/gt/gt mice show an embryonic lethal phenotype. In this study, we have traced the expression pattern of Dlc1 gene during the embryonic developmental stages of Dlc1 wt/gt mouse using the X-gal reporter gene cassette present in the gt vector. Methods: The Dlc1/wt/gt embryos were cryosectioned, and stained with X-gal for organ and tissue specific expression of Dlc1-Xgal transgene. The organ specific expression of DLC1 protein isoforms during developmental stages were studied using antiDLC1 antibodies which could detect all 3 protein isoforms in western blot. The DLC1 protein isoforms expression was correlated with the relative expression of mrRNAs. Results: The Dlc1 gene expression is strongly associated with the development of embryonic heart, the blood vasculature of the placenta and the embryo, somatogenesis, limb organogenesis and brown fat. We have also identified expression of 3 DLC1 protein isoforms in the embryonic heart at 10-12dpc. Conclusion: This result indicates that expression of Dlc1 gene plays a critical role in the organogenesis process during mouse embryonic development. The tissue specific expression of DLC1 protein isoforms shed new insight in the complex regulatory and functional role of the gene.


Unhanded access to model organisms is of paramount importance to advancing the research objectives of the scientific community. To facilitate this access, the Mouse Repository at the non-profit Jackson Laboratory has served as a centralized resource that has distributed and preserved mouse models for over a half century. Over 5,600 strains are available, and more than 500 new strains are imported every year. While all are archived as cryopreserved stocks, more than 1,500 are currently readily available as mice from live colonies. Here we focus on genetic models of human neurodegenerative diseases.

In collaboration with the Michael J Fox Foundation, the repository is developing an extensive set of mouse models related to Parkinson’s disease, including transgenes expressing mutant human alpha-synuclein (SNCA) or Park2 (Parkin), as well as new LRK22 BAC transgenic lines. Targeted mutations of many relevant genes, including Snca, Sncb, Sncb, Pld2, Park2, Park7, and Lrrk2 are also available. Available Alzheimer’s disease strains include more than 40 different models carrying mutations in 14 different genes directly relevant to familial AD. A few select Alzheimer’s models are available as aged mice. The repository also distributes a large set of Huntington’s and other repeat-expansion disease models. A recent focus has been very precise “research tools” strains that express CRE, GFP or effector molecules in specific neuronal subsets. The Repository maintains a searchable online database where strain descriptions can be accessed (www.jaxmice.jax.org/query). Submission of a mouse model to the JAX repository fulfills NIH requirements for sharing of resources in an efficient and cost-effective manner. An online form is available for researchers wishing to donate strains (www.jax.org/gre/index.html).

Support for this project has been provided by the NIH, HHIIM and private charitable foundations.

197B Identification of gene variants modifying obesity and incidence of type 2 diabetes by conventional positional cloning strategies in mice. Stephan Scharnegg, Heike Vogel, Alexandra Chadta, Reinhart Kluge, Hans-Georg Joost, Annette Schurmann. Experimental Diabetology and Pharmacology, German Institute of Human Nutrition Potsdam-
Rehrbrueck, Germany.

Objectives: Obesity and type 2 diabetes are complex and polygenic diseases. In the last years, several gene variants predisposing for these disorders were identified in humans by large genome-wide association studies. However, the mechanisms connecting the variants with the disease phenotype are poorly understood. To bridge this gap inbred strains of mice can be used as a powerful tool. Mouse strains allow the generation of suitable crossing models for the mapping and characterization of disease genes. Methods: New Zealand Obese (NZO) mice, which develop a polygenic disease pattern of obesity, insulin resistance and type 2 diabetes, were crossed with the two lean inbred strains C57BL/6 and SJL and the offspring of a F2 generation and a backcross generation, respectively, was characterized for different metabolic parameters in order to perform linkage analyses. Major QTL were confirmed and narrowed down by breeding of recombinant-congenic strains. Responsible gene variants within the critical regions were finally identified after sequencing and expression profiling. Results: In addition to several small susceptibility loci, three major QTL for obesity and/or diabetes on Chromosomes 1 (Nobq3), 4 (Nidd/SJL) and 5 (Nobq4) were identified. After breeding of recombinant-congenic lines, the responsible gene variants for Nobq1 and Nidd/SJL could be identified (Tbc1d1, Zfp69). The QTL Nobq3 was reduced to a critical fragment of 2.2 Mb containing 43 genes. Interestingly, this region maps to a QTL hotspost designated as Qrta (Mozzoli et al., PLoS Genetics 4, 2008, e1000260). Conclusions: The combination of (i) crossbreeding strategies, (ii) generation of recombinant congenic mouse lines and (iii) sequencing plus expression profiling is a very promising strategy to identify susceptibility genes for obesity and type 2 diabetes. Detection of new QTL and positional cloning of the responsible gene variants are important tools to analyze complex traits.

198C

Maternal age affects the risk of congenital heart disease in a mouse model. Claire E. Schulkey, Suk D.Regmi, Patrick Y. Jay. Departments of Pediatrics, Computational and Systems Biology, Washington University, St. Louis, MO.

Background: Congenital heart disease is a leading cause of death in children and in a complex multifactorial basis despite the usual investigative approach as a simple mendelian disorder. Epidemiologic studies report an association of maternal age and heart defects independent of chromosomal aneuploidy. Heterozygous mutations of the cardiac transcription factor Nkx2-5 cause heart defects in man and mouse. While mapping genetic modifiers of the Nkx2-5 mouse phenotype, we discovered a similar relationship between maternal age and the incidence of ventricular septal defects.

Methods: We collected ~5000 F1 mice from an F2 intercross of the inbred strains C57BL6 and FVB/N. Genotypes, demographic information, i.e., maternal and paternal age and litter size, and phenotypes were recorded. SNP marker data were collected for all affected and a subset of normal Nkx2-5+/- animals. In a separate set of experiments ovaries were surgically transferred between old and young females to localize the maternal age effect to the oocyte or uterine milieu.

Results: Multiple logistic regression analysis revealed a significant effect of maternal age but not paternal age or litter size on the incidence of ventricular septal defects caused by Nkx2-5 mutation. For example, Nkx2-5 homozygous offspring of old mothers (>300 days old) showed a 2-3 fold higher incidence than young mothers (<100 days old). The effect was not related to any genetic polymorphism or chromosomal aneuploidy, based on the analysis of SNP genotype data in the offspring. In ovarian transfer experiments, the incidence of VSD was either higher or low when young or old ovaries were transplanted into old or young recipient mothers, respectively.

Conclusions: Older maternal age increases the risk of congenital heart disease in our mouse model. The effect appears to depend upon genetic predisposition to a heart defect, i.e., Nkx2-5 mutation, but not on copy number variation. Maternal age is a marker of a biological factor in the mother that interacts with cardiac development pathways in the embryo.

199A

Epistatic interactions in wild-derived mice identify evolutionarily preserved amino acids that characterize the adaptor protein TIRAP as an inhibitor of TLR-mediated signaling. Stephen A. Schworer1,2, James R. Conner1,2, Irina I. Smirnova1, Alexander Poltorak1. 1) Department of Pathology, Graduate Program in Immunology, Tufts University, 2) Sackler School of Biomedical Sciences, Boston, MA; 2) Medical Scientist Training Program, Tufts University, Boston, MA.

The Institut Clinique de la Souris (ICS) is a technology platform that provides a comprehensive set of highly specialized mouse services to scientists from academia and industry. The ICS combines the capacity of generating mutant mice on a large scale with a high-throughput and comprehensive phenotypic analysis of mice. The ICS phenotyping platforms are adapted for the study of genetically engineered mouse models, as well as for pharmacological and toxicological studies, allowing better understanding of human diseases and their underlying physiological and pathological basis. The ICS has successfully established a mouse model of obesity, insulin resistance and type 2 diabetes in B6D2F1 mice, which develops a polygenic disease pattern of obesity, insulin resistance and type 2 diabetes (Obese (NZO) mice, which develop a polygenic disease pattern of obesity, insulin resistance). These models can be used to study the development of the yolk sac and placenta. Interestingly, the phenotype of Obese (NZO) mice, which develop a polygenic disease pattern of obesity, insulin resistance and type 2 diabetes, was confirmed in a separate set of experiments ovaries were surgically transferred between old and young females to localize the maternal age effect to the oocyte or uterine milieu.

Results: In ovarian transfer experiments, the incidence of VSD was either higher or low when young or old ovaries were transplanted into old or young recipient mothers, respectively.

Conclusions: Older maternal age increases the risk of congenital heart disease in our mouse model. The effect appears to depend upon genetic predisposition to a heart defect, i.e., Nkx2-5 mutation, but not on copy number variation. Maternal age is a marker of a biological factor in the mother that interacts with cardiac development pathways in the embryo.

200B

comprehensive phenotyping of mouse models. MOHAMMED SELLOUM, TANIA SORG, ABDEL AYADI, ELODIE BEDU, MARIE-FRANCE CHAMPY, ROY COMBE, CLAIRE E. SCHULKEY, SUK D. REGMI, PATRICK Y. JAY. Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University, Ithaca, NY.

Background: Mutual information is defined by the probability of a disease state given the presence of a specific phenotype. For example, a Trim28 hypomorphic allele reveals differential requirements of KRAB zinc finger proteins during mouse embryonic development.

Methods: Many mouse models of human disease are used to investigate genetic and environmental factors that contribute to disease pathogenesis. Mouse strains allow the generation of suitable crossing models for the mapping and characterization of disease genes. Methods: New Zealand Obese (NZO) mice, which develop a polygenic disease pattern of obesity, insulin resistance and type 2 diabetes, were crossed with the two lean inbred strains C57BL/6 and SJL and the offspring of a F2 generation and a backcross generation, respectively, was characterized for different metabolic parameters in order to perform linkage analyses. Major QTL were confirmed and narrowed down by breeding of recombinant-congenic strains. Responsible gene variants within the critical regions were finally identified after sequencing and expression profiling.

Results: In addition to several small susceptibility loci, three major QTL for obesity and/or diabetes on Chromosomes 1 (Nobq3), 4 (Nidd/SJL) and 5 (Nobq4) were identified. After breeding of recombinant-congenic lines, the responsible gene variants for Nobq1 and Nidd/SJL could be identified (Tbc1d1, Zfp69). The QTL Nobq3 was reduced to a critical fragment of 2.2 Mb containing 43 genes. Interestingly, this region maps to a QTL hotspost designated as Qrta (Mozzoli et al., PLoS Genetics 4, 2008, e1000260). Conclusions: The combination of (i) crossbreeding strategies, (ii) generation of recombinant congenic mouse lines and (iii) sequencing plus expression profiling is a very promising strategy to identify susceptibility genes for obesity and type 2 diabetes. Detection of new QTL and positional cloning of the responsible gene variants are important tools to analyze complex traits.

201C


Mammalian genomes contain over 300 Zinc finger proteins with Kruppel-associated box (KRAB) domains. It has been proposed that the functions of all KRAB zinc finger family members are mediated by TRIM28 (also known as TIF1)I, KAP1 or KIRP1), a universal co-repressor protein that recruits chromatin modifying enzymes. Although the early lethality of Trim28 knockout mice, which arrest prior to gastrulation (embryonic day 5.5) has highlighted an essential role for TRIM28 in early embryonic development, requirements for TRIM28 during later stages of embryogenesis are not well understood. Here we show that chatwo, a recessive ENU-induced mutation in the mouse, creates missense mutations in the bdomaion of TRIM28 and causes embryonic lethality at embryonic day 8.5. Complementation analysis with a Trim28 knockout allele indicates that the chatwo mutation generates a hypomorphic Trim28 allele. chatwo embryos display strong morphogenetic defects, including abnormalities in embryonic convergent extension and in the development of the yolk sac and placenta. Interestingly, the phenotype of chatwo mutants is similar to that of mouse mutants in the KRAB zinc finger protein ZFP568, suggesting that chatwo disrupts ZFP568-specific functions of TRIM28. Interaction of TRIM28 with ZFP568 was confirmed by yeast two hybrid, immunoprecipitation and co-localization of the two proteins in cell lines. Furthermore, we show that Trim28 is a required cofactor of ZFP568 in embryonic tissues to regulate embryo morphogenesis. These results provide molecular and genetic evidence that the ZFP568-TRIM28 interaction is important for embryonic morphogenesis, and suggest that TRIM28 is a major mediator of ZFP568 functions during embryonic development.

202A

Ahnak ablation results in lean mice with enhanced insulin sensitivity. Jae Hoon Shin1, Il Yong Kim1, Yo Na Kim1, Ji Won Choi1, Seo Hyun Lee1, Kyung Jin Roh1,2, Mi Ra Son1, Cheol Soo Choi1,2, Yoon Soo Bae1, Je Kyung Seong1. 1) Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea; 2) Seoul National University College of Medicine, Seoul, Korea; 3) Medical and Health Sciences Research Institute, Seoul National University College of Medicine, Seoul, Korea; 4) Department of Pediatrics, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea; 5) Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea.

Ahnak ablation results in lean mice with enhanced insulin sensitivity. Jae Hoon Shin1, Il Yong Kim1, Yo Na Kim1, Ji Won Choi1, Seo Hyun Lee1, Kyung Jin Roh1,2, Mi Ra Son1, Cheol Soo Choi1,2, Yoon Soo Bae1, Je Kyung Seong1. 1) Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea; 2) Seoul National University College of Medicine, Seoul, Korea; 3) Medical and Health Sciences Research Institute, Seoul National University College of Medicine, Seoul, Korea; 4) Department of Pediatrics, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea; 5) Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea.
recording electrodes on the scalp and calibrated freefield broadband clicks and tonebursts delivered in 5dB steps. We test at least four mice per mutant line, and the measurement
signs of many diseases, including deafness and balance defects. To detect hearing impairment, we record Auditory Brainstem Responses (ABRs) from anaesthetised mice using pin
Mouse Genetics Project, using the resource of targeted mouse ES cells to generate mouse lines with mutations in known genes. We have screened over 330 new mutant lines for
known to have a hearing impairment beforehand and were included as positive controls. All nine showed raised thresholds as expected. Among the remaining lines, we have
To elucidate the role of AHNAK on adipogenesis, we generated

207C
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Characterisation of Laterality Defects in the K27 ENU Mutant. Louise A. Stephen, Karen Mitchell, Kathryn E. Hentges. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

Heterotaxy is a rare but devastating condition that affects roughly 11 live births in the USA every year, and is a suspected cause of many failed pregnancies. Very little is known about the aetiology of this disease due to a variety of causes. Heterotaxy leads to the misarrangement of internal organs, most often causing congenital cardiac defects. Research into this syndrome concentrates mainly on cardiac development and the establishment of left-right polarity within the developing embryo. Our lab has taken a forward genetics approach to discovering novel genes required for appropriate cardiac left-right polarity by characterising the effects of the K27 mutation, which disrupts cardiac asymmetry. The K27 mutation was identified in an ENU mutagenesis screen. The mutation results in a homozygous lethal phenotype by embryonic day 12. The K27 phenotype includes a developmental delay and abnormal cardiac morphology including a reversal of cardiac looping. In situ hybridisation, histology and immunohistochemistry have been used alongside ultrasound to characterise these phenotypes. We have investigated a series of left-right mutants using in situ hybridisation and quantitative real time PCR to identify abnormalities in specification of the left-right axis. Motivic mapping has refined the K27 candidate region to a 5 Mb interval on mouse Chromosome 11 containing over 100 genes.

Several steps have been taken to identify possible candidate genes and a mixture of reverse transcription PCR, quantitative real time PCR and sequencing have eliminated approximately one-third of the candidates, including all those linked to cardiac and left-right development. Work to identify the mutation definitively using high-throughput sequencing is currently ongoing. Continued analysis of the K27 mutants will identify the gene mutated in the K27 mouse and define the role that this gene plays in cardiac development and left-right asymmetry. Combined these studies will identify a novel gene that contributes to our understanding of cardiac congenital birth defects and left-right axis formation.

208A

An F1 strain survey to facilitate mapping of aganglionosis modifiers in the Sox10^flox^>Hirschsprung disease model. T. Stobdan, JM Dekeyser, AW Nickle, KM Bradley, JR Smith, EM Southard-Smith. Genetic Medicine, Vanderbilt University, Nashville, TN.

Hirschsprung disease (HSCR) is diagnosed clinically by the absence or reduction of enteric ganglia, in the distal gastrointestinal (GI) tract. Cumulative evidence among human families and mouse models indicates that HSCR is the consequence of multiple gene interactions that produce the variable penetrance and loss of enteric ganglia. The Sox10^flox^ model of HSCR exhibits phenotype in heterozygotes that is influenced by genetic background and thus is a valuable model for identifying the genes that predispose to HSCR. Prior analyses using a traditional F1 intercross with C57BL6J and C3H/HeJ strains identified 5 loci that modified aganglionosis in Sox10^flox^>F2 progeny. To further refine, we undertook a strain survey to identify those inbred strains that dichotomized the aganglionosis phenotype the most. We took the novel approach of examining phenotypes in F1 Sox10^flox^ mutants. Crosses of the C57BL6J/Sox10^flox^ line with 35 distinct inbred strains were performed. Whole-mount AChE staining of GI tracts from F1 pups at P7-P10 was used to assess enteric neurite growth, gut length (TGL), intestinal ganglion formation (sox10 immunostaining) and hypoganglionosis were recorded for all F1 pups. Among the strains evaluated, Sox10^flox^>F1 pups from crosses with A/J were most severely affected while crosses with PWD/PJd were the least affected. Haplotypic association (HA) mapping was implemented to identify new modifiers for aganglionosis across the 35 strains. Significant regions were identified on Chr 1, 2, 4, 5, 12, 14 & 17 at P<1x10^−6-. Significant intervals observed in previously mapped modifier regions of F2 mapping study are being compared to the regions identified in HA analysis. This is the first strain survey for aganglionosis modifiers and has laid the groundwork for identification of novel genes that modulate penetrance and severity of aganglionosis in HSCR.

209B

Pierce1, a novel TRP53 target gene required for normal DNA-damage responses. Young Hoon Sung, Sungil Devkota, Jeehoon Lee, Han-Woong Lee. Department of Biochemistry, and YLARC, Yonsei University, Seoul, Korea.

Retinoblastoma (Rb) is an important tumor suppressor gene and modulates TRP53 activity by regulating the expression of an E2F target gene, Cdkn2a (p19^ARF^). In order to identify novel genes regulated by Rb/E2F, we analyzed the gene expression pattern of Rb-deficient mouse embryonic fibroblasts (MEFs) and found that expression of diverse genes was altered in Rb-deficient MEFs. Among these genes, we identified that Pierce1, a novel transcript was up-regulated in Rb-deficient MEFs. Although it was encoded as an E2F target gene deregulated upon Rb deficiency, Pierce1 promoter did not respond to E2F1 in Cdkn2A^−/−cell lines. Rather, Pierce1 promoter was strongly activated by TRP53 via two cis-elements. In support, the expression of Pierce1 was induced by genotoxic stresses in wild-type MEFs, but not in TRP53-deficient MEFs. In addition to the transcriptional regulation, Pierce1 protein was post-translationally stabilized by ultraviolet C (UVC) irradiation, and UVC-activated ATR signaling suppressed pro-apoptotic degradation of Pierce1 protein. These results consistently suggest that Pierce1 should be involved in transducing the DNA-damage response. In fact, knockdown of Pierce1 compromised the checkpoint response of wild-type MEFs to UVC irradiation, accompanying the diminished expression of TRP53 target genes. Taken together, our data suggest that Pierce1 is an important TRP53 target gene and contributes to normal DNA-damage response. Therefore, Pierce1 may play crucial roles in maintaining genomic integrity against genotoxic stresses, including UVC irradiation. Furthermore, we have established a mouse model deficient in Pierce1 and are examining their phenotype associated with tumorigenesis and DNA-damage response.

210C

Modifiers of cardiac muscle fibrosis and function in a mouse model of muscular dystrophy. Kayleigh A. Swaggart1, Ahlike Heydemann2, Gene H. Kim1,2, Jenan Holley-Cuthrell1, Kayleigh A. Swaggart1, Elizabeth M. McNally1,2,1) Department of Human Genetics, The University of Chicago, Chicago, IL; 2) Department of Physiology and Biophysics, University of Illinois at Chicago, Chicago, IL, 3) Department of Medicine, The University of Chicago, Chicago, IL.

Mutations in single genes lead to inherited forms of cardiomyopathy and muscular dystrophy. Despite identical gene mutations, there is often significant variability in these Mendelian disorders. In human patients the age of onset and severity of cardiac and skeletal muscle disease progression are highly variable. In mice, this phenotypic variability is modeled by X-linked muscular dystrophy. Among these genes, we identified that Pierce1 c, a novel transcript was up-regulated in Rb-deficient MEFs. Although it was encoded as an E2F target gene deregulated upon Rb deficiency, Pierce1 promoter did not respond to E2F1 in Cdkn2A^−/−cell lines. Rather, Pierce1 promoter was strongly activated by TRP53 via two cis-elements. In support, the expression of Pierce1 was induced by genotoxic stresses in wild-type MEFs, but not in TRP53-deficient MEFs. In addition to the transcriptional regulation, Pierce1 protein was post-translationally stabilized by ultraviolet C (UVC) irradiation, and UVC-activated ATR signaling suppressed pro-apoptotic degradation of Pierce1 protein. These results consistently suggest that Pierce1 should be involved in transducing the DNA-damage response. In fact, knockdown of Pierce1 compromised the checkpoint response of wild-type MEFs to UVC irradiation, accompanying the diminished expression of TRP53 target genes. Taken together, our data suggest that Pierce1 is an important TRP53 target gene and contributes to normal DNA-damage response. Therefore, Pierce1 may play crucial roles in maintaining genomic integrity against genotoxic stresses, including UVC irradiation. Furthermore, we have established a mouse model deficient in Pierce1 and are examining their phenotype associated with tumorigenesis and DNA-damage response.

211A


Down syndrome (DS) is a disorder caused by the triplication of human chromosome 21. The resultant gene dosage imbalance has been linked to mental retardation, cardiac and neuronal defects as well as craniofacial and appendicular skeletal abnormalities. In particular, shorter humerus and femur lengths have been observed in second trimester DS fetuses, and though documented, very little is known about the developmental origins of the appendicular skeletal defects associated with DS. Using the Ts65Dn mouse model for DS, we evaluated skeletogenesis in embryonic day (E) 13.5 -14.5 embryos. Through whole mount skeletal staining, we observed a significant increase in the humerus length of Ts65Dn embryos as compared to their euploid littermates at E13.5. Although contradictory to findings for the overall smaller skeletal size observed in neonatal and adult mice, we attribute the increased length observed to the poorly defined structure of the cartilaginous skeleton during precartilage condensation. Collagen II, osteopontin and collagen X are expressed in the cartilaginous appendicular skeleton at E13.5-14.5 and act as indicators of skeletogenesis in murine development. Quantifying specific protein expression in the femur and humerus during skeletogenesis using immunohistological analysis identifies variability between Ts65Dn and euploid bone formation at this critical stage of development. Furthermore, we aim to analyze chondrocyte apoptosis, an event in skeletal development that triggers nucleation for matrix calcification at E13.5-14.5, using TUNEL (TdT-mediated dUTP Nick End Labeling) staining. Examining the developmental basis of abnormal appendicular skeletal phenotypes would provide information regarding phenotypic variability, as well as possible therapeutic strategies, to aid individuals with Down syndrome.

212B

Genetic dissection of the early susceptibility of MBT/PAS mice to Rift Valley Fever. Satoko Tokuda1, Tânia Zaverucha do Valle1, Agnès Billecocq2, Claudia Pommerenke3, Robert Gelfer1, Klaus Schughart1, Michèle Bouloy2, Xavier Montagutelli1, Jean-Jacques Panthier1. 1) Mouse Functional Genetics Unit, CNRS URA 2578, Institut Pasteur, Paris, France; 2) Molecular Genetics of Bunyaviruses, Institut Pasteur, Paris, France; 3) Array Facility/Cell Biology, Helmholtz Centre fur Infection Research, Braunschweig, Germany;

POSTER: Mouse models of human disease

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4) Experimental Mouse Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany. Rv11 Valley Fever virus (RVFV) is responsible for an arthropod-borne zoonosis. In humans, the disease is usually benign although a small percentage of patients exhibit complications, characterized by hepatitis with hemorrhage and meningoen cephalitis. An influence of host genetic factors has been suggested but remains unidentified. We previously demonstrated that a wild-derived inbred strain, MBT/Pas mice (Mus m. musculus), is highly susceptible to experimental infection with RVFV virus ZH548 strain, compared to BALB/cByJ mice. To dissect the genetic components underlying the susceptibility of MBT/Pas mice to RVF, 546 (BALB/cByJ × MBT/Pas) F2 animals were analyzed and demonstrated for the first time on chr 2 and 11: Rv11 Valley fever susceptible loci (Rvfs1) 1 and (Rvfs2), respectively. An additional locus, Rvfs3 (suggestive), was identified on chr 5. Congenic mice for each locus were generated on the BALB/c background and challenged with ZH548 RVFV. We observed that mice congenic for Rvfs2 (CMBT-Rvfs2) died significantly earlier than BALB/c. We also investigated the susceptibility by in vitro infection of peritoneal macrophages. RVFV titers were significantly higher in CMBT-Rvfs2 macrophages than in BALB/c macrophage supernatants, suggesting increased RVFV replication in CMBT-Rvfs2 macrophages. Gene expression profiling in RVFV-infected macrophages from CMBT-Rvfs2 and BALB/c is underway to identify the gene underlying Rvfs2 and characterize the mechanism of resistance to RVF.

213C Interactions of the Hybrid sterility 1 gene, Prdm9, Zdenek Trachut1, Petr Flach1, Yasuhisa Matsui2, Jiri Forejt1. 1) Department of Mouse Molecular Genetics and Center for Applied Genomics, Inst. Molecular Genetics Acad. Sci., Czech Republic; 2) Cell Research Center for Biomedical Research, IDAC, Tohoku Univ., Sendai, Japan.

The Dobzhansky-Muller (DM) model of hybrid incompatibilities explains speciation by impeding epistatic interactions. These results led to hybrid fitness reduction because the combination of the diverged alleles of the interactors did not pass through natural selection. Although the mechanism of speciation is of great interest, only a few DM incompatibilities have been characterized in animals at a gene level, all in Drosophila. The Hybrid sterility 1 gene (Hst1) participates in meiotic arrest of spermatogenesis in the offspring of males Mus m. domesticus origin (carrying the Hst1 allele crossed to females coming Mus m. musculus. These hybrid males carry no sperm, while the F1 hybrids from the reciprocal cross display only reduced sperm count. Previously, we have identified Hst1 with Prdm9 (PR-domain containing 9), encoding histone 3 lysine 4 monomethy transferase. The gene is necessary for successful meiosis, as the Prdm9 germ cells arrest at pachynema.

To reveal the DMs involving Hst1 in mouse hybrid sterility, we prepared intersubspecific F1 males carrying the null Hst1 allele using Prdm9 knock-out and phenotyped them along with littermate controls. The knock-out partially rescued fertility in otherwise azoospermic hybrids. Given that the animals bearing Prdm9 may participate in yet another DM, all other DMs also play a role. In the reciprocal intersubspecific hybrids, the null Hst1 allele rescued full fertility. Thus, Hst1 may participate in the first time in PRDM9’s causal role in the modification of cancer phenotypes.

Identification of Grb10 as a Modifier of MPNST growth in the NCis Mouse Model of NF1 Jessica C. A. Van Schaick1, Christina DiFabio1, Sandra Burket1, Keiko Akagi1, Kelly H. Smith1, Jessica Walrath1, Robert Tuskal1, Karlyne Reilly1. 1) National Cancer Institute, Frederick, MD; 2) The Ohio State University, Columbus, OH.

The current study aimed to identify modifier genes of malignant peripheral nerve sheath tumors (MPNSTs) in the Nf1;p53cis (NPcis) mouse model of NF1. Previous studies have shown that the incidence of MPNST development in the NPcis mouse model is affected by the parental transmission of the mutant chromosome 11. In this study microarray analysis was used to examine gene expression differences between MPNST primary tumors derived from NPcis mice varying in inheritance of the NPcis chromosome from the mother (NPcis maternal) or father (NPcis paternal). Grb10 was found to be more highly expressed in NPcis paternal MPNSTs; Zrsr1 was found to be more highly expressed in NPcis paternal MPNSTs. qPCR was used to validate both gene expression differences. We focused first on Grb10 due to its role as a cytoplasmic signaling adapter protein. Fluorescence in situ hybridization was used to examine the presence of Grb10 on chromosome 11. Grb10 was found to be lost more frequently in NPcis paternal MPNST cell lines, most likely by contributing to the decrease in Grb10 gene expression in the paternal germ line. In addition, Grb10 is paternally imprinted in the periphery of the mouse, therefore we examined Grb10 isospecific expression and found paternally and maternally expressed isospecifics expressed in the MPNSTs. Due to these results we are examining the loss of imprinting is contributing to tumorigenesis. MPNST cell lines stably overexpressing Grb10 were generated and found to have a significant decrease in proliferation. Finally we have generated NPcis;Grb10cis mutant mice. Our studies indicate that NPcis;Grb10cis maternal mice have a significant increase in MPNST incidence and a significant decrease in survival. Additionally, NPcis;Grb10cis maternal MPNSTs have an increase in tumor size and mitotic index. These results identify Grb10 as the first modifier of MPNSTs by acting in a tumor suppressive manner and demonstrate for the first time that Grb10’s causal role in the modification of cancer phenotypes.

213B The current progress and future plan of JAPAN MOUSE CLINIC in BREIN RIKEN, Shigebaru WAKANA1, Tomohiko SUZUKI1, Tamio FURUISE1, Hideki KANEDA1, Kimio KOBAYASHI1, Ikuo MIURA1, Hiromi MOTEGI2, Hideaki TOKI2, Maki INOUE2, Osamu MINOWA2, Tetsuo NODA3, Kazunori WAKA3, Nobuhiko TANAKA3, Hiroshi MASUYA1. 1) Japan Mouse Clinic, RIKEN BRC, Tsukuba, Ibaraki, Japan; 2) Team for Advanced Development and Evaluation of Human Disease Models, RIKEN BRC, Tsukuba, Ibaraki, Japan; 3) Technology and Development Unit for Knowledge Base of Mouse Phenotype, RIKEN BRC, Tsukuba, Ibaraki, Japan.

The Japan Mouse Clinic (JMC) has been launched in 2008, and aims its tools to evaluate comprehensive and detailed phenotypic characteristics for various mouse resources based on standardized operating procedures. We have already performed approximately 93 strains holding common inbred strains, wild derived strains, genetically-engineered strains, and ENU mutant strains on JMC comprehensive phenotyping platform. The large phenotypic data set of the 45 mutant or genetically modified mouse strains, we analyzed the frequency of statistically significant difference among genotypes on ca. 220 parameters in each strain. And we summarized the frequencies of significance are 1.5% in male, 1.2% in female. The frequency is far smaller than that of single parameter. And we identified some significant differences in the specific strain. The next step we have a plan to take part in the IMPC (International Mouse Phenotyping Consortium) program. We will harmonize the different phenotyping procedures between IMPC and JMC, and enhance JMC phenotyping platform. Furthermore we have another plan to analyze gene-environmental interactions in the areas nutrition, facility condition, and stress. This plan will help us to study genetic predispositions for mouse models of human disease.

216C A protein at the intersection of coat-color biology and brain disease: Tsg101 ablation causes neurodegeneration in adult mice. Will P. Walker1, Caroline We2, Elena Sviderskaya1, Kay-Uwe Wagner4, Teresa M. Gunn1. 1) McLaughlin Research Institute, Great Falls, MT; 2) Harvard University Department of Neuroscience, Cambridge, MA; 3) St. George's University of London, London, UK; 4) Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE.

Mouse with null mutations for either the type I transmembrane protein, attractin (Arr) or the E3 ubiquitin ligase, mahogunin ring finger 1 (Mgrn1) exhibit a remarkably similar pleiotropic phenotype characterized by black coat color, central nervous system (CNS) demyelination, and spongiform neurodegeneration. This striking phenotypic overlap suggests that ATRN and MGRN1 act together in a conserved molecular pathway that is active both in the hair follicle and the CNS, and that the pigmentation and neurological phenotypes in these mutants, while superficially dissimilar, are likely to be mechanistically related. MGRN1 has been implicated in the regulation of TSG101, which regulates mono ubiquitinated transmembrane proteins on endosomes and sorts them into the lysosomal trafficking pathway. Investigations in our lab support a model in which ATRN and MGRN1 act in concert to promote lysosomal trafficking and degradation of the melanocortin 1 receptor (MCIR), thereby explaining the color coat defects of Arr and Mgrn1 mutant mice. These results led us to hypothesize that impaired endo/lysosomal protein sorting in the brain could underlie the CNS abnormalities seen in Arr and Mgrn1 mutant mice. We tested this hypothesis using an inducible conditional gene ablation strategy to knock out Tsg101 in forebrain neurons of adult mice. Loss of Tsg101 effectively models endo/lysosomal protein sorting dysfunction and leads to pronounced neurological defects; indicating that disruption of endosome-to-lysosome protein sorting may be an important primary pathological mechanism in some types of neurodegenerative disorders.

217A Quantitative trait locus analysis of intestinal polyposis in the C3H/HcJ genome. Xiang Wang1, Stephani Nnadi2, Reviat Korakar2, Karl Bromam3, Arthur Buchberg3, Linda Siracusa1. 1) Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA 19107; 2) Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN 55455; 3) Department of Biostatistics & Medical Informatics, University of Wisconsin-Madison, Madison, Wisconsin 53706.

Genetic background can dramatically affect small intestine and colon polyp development in the ApcMin mouse model. The Modifier of Min (Mom1) locus is the first modifier locus of intestinal polyp formation identified and accounts for ~50% of the variation in polyp multiplicity between susceptible C57BL/6J (B6) mice and resistant C3H/HcJ (C3H) mice. By using reciprocalMom1 congenic lines, the effect of the Mom1 locus was eliminated and we demonstrated that the C3H strain has modifier loci (other than Mom1) that suppress intestinal polyp multiplicity. To identify these new modifier genes, we carried out a quantitative trait locus (QTL) cross, in which resistant C3H.B6 Mom124176 congencic
mice were intercrossed with susceptible B6 Mmumu+/+ mice to generate F1 hybrids; the F1 hybrid mice were then backcrossed to B6 Mmumu-/- mice. The N2 offspring were either heterozygous (CBA/J x C57BL/6J)F1 x CBA/J or homozygous (DBA/2J x C57BL/6J)F1 x DBA/2J and were taken at 120 days of age. The haploinsufficient number, size and position were determined. The segregation of alleles across the genome was determined and correlated with polyp phenotypes by R/qtl analysis. A square-root transformation was used to stabilize the variance of polypl number in the small intestine and colon. The results revealed several unlinked modifier loci. In addition, a subset of modifier loci appear to have gender-specific and tissue-specific effects. We have evaluated the effects of these loci, singly and in combination. Experimental crosses between different modifier loci are under further investigation. The results may reveal different pathways controlling intestinal polyposis. Research supported in part by NCI grants to LDS and AMB.

218B
A novel Sox10 modifier locus identified in a sensitized ENV: mutagenesis screen. Dawn Watkins-Chow1, Karen Leed1, Raymond Mulllen2, Arturo Incio1, Cecilia Rivas1, William Pavan1. 1) Genetic Disease Research Branch, NIHGB, NIH, Bethesda, MD; 2) ES Cell and Tg Mouse Core, NIHGB, NIH, Bethesda, MD.

Melanocytes are specialized, neural crest-derived cells responsible for pigment production in the skin. Disruption in neural crest development can result in altered pigmentation in skin and hair and can be associated with debilitating diseases including deafness, blindness, cleft lip, congential megacolon, and albinism. Collectively, these diseases are referred to as neurocristopathies. Because genetic background is known to affect the severity of neurocristopathies in both humans and mice, we previously established an enhancer screen to identify mutations in the phenotypic severity of Sox10 haploinsufficiency (Sox10+/+) phenotype. This is a recessive model of human neurocristopathies. The goal of this mutagenesis screen is to identify previously uncharacterized pathways affecting melanocyte development and to generate new models relevant for dissecting human disease etiology. From analysis of 600 pedigrees in a dominant screen, we identified the 5 dominant modifiers of the Sox10 phenotype (Mos1-5). Mos3 exhibits a unique, Sox10-dependent, semi-dominant phenotype. Mos3/Mos3 homozygotes exhibit embryonic lethality, but Mos3+/- heterozygotes appear indistinguishable from their wild type littermated. On a Sox10+/+ background, heterozygosity for the Mos3 mutation causes white head spotting that is never observed in Sox10+/+ mice. Embryonic analysis shows that Mos3 causes this synergistic reduction in cranial crest-derived melanoblasts by embryonic day 12.5, before a synergistic reduction in trunk melanoblastlos is observed. This suggests that compared to other spotted mutants, Mos3 more severely affects the cranial crest and may reveal a novel pathway affecting melanoblast development. Further comparative analysis of phenotypes identified in our screen will contribute to our understanding of genome function and provide additional disease models for human neurocristopathies and melanomatosis progression.

219C
Understanding the genetics of vesico-ureteric reflux using inbred mouse models. Christine L. Watt1, Inga J. Murawski1, Jasmine El Andalousi1, Rita W. Maina1, Indra R. Gupta1, 2) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 2) Department of Pediatrics, McGill University Health Center, Montreal, Quebec, Canada.

Vesico-ureteric reflux (VUR) is a congenital defect of the urinary tract that results in the retrograde flow of urine from the bladder to the kidney. VUR affects 1% of the population, predisposing them to recurrent urinary tract infections, hypertension and end stage renal disease. We have previously shown that the C3H/HeJ mouse has a 100% incidence of VUR. Genetic characterization of the phenotypic expression and maps to a 22cM susceptibility locus on Chromosome 12: Vurm1. The CBA/J, DBA/2J and AKR/J have a 55%, 38% and 28% incidence of VUR, respectively. In contrast, the C57BL/6J mouse has a 0% incidence of VUR. Complementation analysis between the C3H/HeJ and other inbred strains suggests Vurm1 also confers VUR susceptibility in these inbred strains. Objective: To validate the Vurm1 locus using other inbred strains and to identify candidate genes that may confer VUR susceptibility. Methods: F1 hybrids derived from crossing a refluxing inbred strain (CBA/J, DBA/2J and AKR/J) and a non-refluxing (C57BL/6J) strain were generated and tested for VUR. These F1 hybrids were then backcrossed to their refluxing parental inbred strain and tested for VUR. DNA from approximately 320 backcross (N2) mice has been extracted and will be genotyped for SNPs spanning the entirety of chromosome 12. Linkage analysis will identify regions of VUR susceptibility and validate Vurm1 locus.

220A
MRC Harwell: Disease Model Discovery, Screening, shard Panogenotyping and Distribution in the International Mouse Phenotyping Consortium (IMPC). Tom Weaver1, Sara Wells1, Martin Fray1, Neil Adams1, Alison Walling1, Paul Potter2, Anne-Marie Mallon2, Nanda Rodrigues2, Steve Brown2, Mark Moore1. 1) Mary Lyon Centre, MRC Harwell, United Kingdom; 2) Mammalian Genetics Unit, MRC Harwell, United Kingdom; 3) National Institutes of Health, USA.

MRC Harwell is an international centre for mouse genetics and is part of the UK Medical Research Council. Our research program and national infrastructure has established large-scale chemical and targeted mutagenesis methodologies and high throughput pipelines for systematically screening mouse mutants for useful phenotypes modelling human disease, and include pipeline tests for - Metabolism: Diabetes, Liver Disease, Bone and Mineral Disorders; Sensory: Deafness & Vision; Neurological: Neuromuscular, Neurodegeneration; Behaviour: Depression, Anxiety; Cardiovascular: Heart structure and function; Respiratory: Lung structure and function; Inflammation: Innate immunity; Reproductive Systems: Sexual development; Embryogenesis: Patterning and Imprinting; Aging: Late Onset Disease; Target Validation and Efficacy: Drug Dosing. To date over 650 models have been identified through these screens. In addition, Harwell is a national archive and distribution centre with many mouse stocks available as frozen embryos or sperm. We facilitate import/export of mouse lines from other countries through a "hub-to-hub" model of collaborating national archives and mouse infrastructures, including the European Mouse Mutant Archive. Harwell infrastructure and resources are available to any academic or commercial research program either through collaboration or fee-for-service. We will present example case studies of these programs, as well as our contribution to a global effort called the International Mouse Phenotyping Consortium (IMPC) which has been created to develop systematically knockout mouse models to study the phenotypic consequences in the resultant mouse strains. This unprecedented program, essentially an Encyclopaedia of Mammalian Gene Function, will provide a step change in our understanding of mammalian physiology, development and pathology.

221B
KOMP312: A Pilot Study to Create and phenotype 312 KOMP Knockout Alleles in Mice. D. West1,2, P. de Jong1, K. Wasson2, E. Engelhard2, K. Lloyd2. 1) Childrens Hospital of Oakland Research Institute, Oakland, CA; 2) Mouse Biology Program, University of California, Davis CA.

The Knockout Mouse Project (KOMP, www.komp.org) is funded to create 5,800 embryonic stem (ES) cells with targeted mutations (knockout first, targeted trap, deletion) for protein coding genes. In concert with the international effort to provide functional annotation for all protein-coding genes in the genome, the NIH plans to fund the creation and phenotyping of 2,500 unique mutants over the next 5 years using the targeted alleles from the International Knockout Mouse Project (KMC). We summarize here a pilot project to create and phenotype 312 mutants using primarily KOMP targeted ES cells (www.kompphenotype.org). The gene selection algorithm emphasizes genes of interest to the scientific community, with no previously characterized null mutants, and with priority given to genes with no annotation. To date we have initiated the analysis of > 800 ES cell lines and germline transmission testing for >300 unique mutant lines with breedable chimeras. Over 160 unique germline-confirmed mutant lines have been generated, and ~70 lines are being bred to homozygosity (HET x HET crosses) for adult phenotyping of TM and T7HOM for each line. Phenotyping emphasizes LacZ expression and histochromically in adults, behavioral testing, and necropsy findings at 50-days-of-age. A compensatory transcriptome analysis on tissues informed by LacZ expression analysis has been initiated in a subset of the mutants bred to homozygosity using Illumina Beadarray assays and RNA-seq. Our experience demonstrates the feasibility of a high-throughput, comprehensive, and high-quality effort to produce KO mice and phenotyping data for all protein coding genes in the genome. Supported by NIH Grants: 3U10HG004080-04S1, 3U42RR024244-03S2.

222C
Gene expression analysis in a murine tuberculosis infection model as a systematic approach to study resistance. Esther Wilk1,2, Galina Shepelkova1, Claudia Pommerenke1, Rudi Alberts1, Alexander Ap2, Klaus Schughart1. 1) Infection Genetics, Helmholtz Center for Infection Research, Braunschweig, Germany; 2) Central Institute for Tuberculosis, Laboratory for Immunogenetics, Moscow, Russia.

Tuberculosis is one of the world’s most threatening infectious diseases. Resistance to treatment and an increasing rate of TB-HIV co-infections underline the need for new strategies to fight this disease. For a better understanding of the pathology and the role of host genetics a unique mouse model of a resistant (A/Sn) and a susceptible (C57BL/6J) strain was used. This heterozygous model is a well-characterized mouse model of human tuberculosis. From analysis of 600 pedigrees in a dominant screen, we identified the 5 dominant modifiers of the Sox10 phenotype (Mos1-5). Mos3 exhibits a unique, Sox10-dependent, semi-dominant phenotype. Mos3/Mos3 homozygotes exhibit embryonic lethality, but Mos3+/- heterozygotes appear indistinguishable from their wild type littermated. On a Sox10+/+ background, heterozygosity for the Mos3 mutation causes white head spotting that is never observed in Sox10+/+ mice. Embryonic analysis shows that Mos3 causes this synergistic reduction in cranial crest-derived melanoblasts by embryonic day 12.5, before a synergistic reduction in trunk melanoblastlos is observed. This suggests that compared to other spotted mutants, Mos3 more severely affects the cranial crest and may reveal a novel pathway affecting melanoblast development. Further comparative analysis of phenotypes identified in our screen will contribute to our understanding of genome function and provide additional disease models for human neurocristopathies and melanomatosis progression.
Cyclin D3 is overexpressed in human breast cancers and compensates for the loss of Cyclin D1 during mammary tumor initiation and progression. The action of multiple genes in tumorigenesis.

Cyclin D1 and D3 are overexpressed in human breast cancer cell lines and primary invasive breast cancers, and Cyclin D3 frequently exceeds the expression of Cyclin D1 leads to a compensatory upregulation of Cyclin D3, which explains why the targeted downregulation of Cyclin D1 in established mammary tumors had no effect on cancer cell proliferation. Cyclin D1 is a suggested molecular target for the treatment of ErbB2-positive breast cancer. The current work addresses whether Cyclin D1 is indispensable for mammary tumor initiation and progression using a breast cancer model in which this cell cycle regulator can be genetically ablated prior to or after neoplastic transformation. Deficiency in Cyclin D1 leads to a compensatory upregulation of Cyclin D3, which explains why the targeted downregulation of Cyclin D1 in established mammary tumors had no effect on cancer cell proliferation. Cyclin D1 and D3 are overexpressed in human breast cancer cell lines and primary invasive breast cancers, and Cyclin D3 frequently exceeds the expression of Cyclin D1 in ErbB2-positive cases. The simultaneous downregulation of both cyclins reduced the proliferation of cancer cells in vitro and decreased the tumor burden in vivo, suggesting that only the combined inhibition of Cyclin D1 and D3 is a suitable strategy for breast cancer therapy.

Cyclin D3 is overexpressed in human breast cancers and compensates for the loss of Cyclin D1 during mammary tumor initiation and progression. Qian Zhang1, Kazuhiro Sakamoto1, Chengbao Chengbao Liu2, Aleata Triplett1, Wan-chi Lin1, Hallgeir Rui2, Kay-Uwe Wagner1. 1) University of Nebraska Medical Center, Omaha, NE; 2) Kimmel Cancer Center, Philadelphia, PA.

Cyclin D1 is a suggested molecular target for the treatment of ErbB2-positive breast cancer. The current work addresses whether Cyclin D1 is indispensable for mammary tumor initiation and progression using a breast cancer model in which this cell cycle regulator can be genetically ablated prior to or after neoplastic transformation. Deficiency in Cyclin D1 leads to a compensatory upregulation of Cyclin D3, which explains why the targeted downregulation of Cyclin D1 in established mammary tumors had no effect on cancer cell proliferation. Cyclin D1 and D3 are overexpressed in human breast cancer cell lines and primary invasive breast cancers, and Cyclin D3 frequently exceeds the expression of Cyclin D1 in ErbB2-positive cases. The simultaneous downregulation of both cyclins reduced the proliferation of cancer cells in vitro and decreased the tumor burden in vivo, suggesting that only the combined inhibition of Cyclin D1 and D3 is a suitable strategy for breast cancer therapy.

How does mouse a-synuclein protect against human A53T mutant a-synuclein toxicity? Dan Zou, Deborah Cabin. McLaughlin Research Institute, Great Falls, MT. a-synuclein (SNCA) is linked to sporadic and familial forms of Parkinson’s disease (PD). Three missense mutations as well as duplication of unmutilated SNCA cause familial forms of PD. SNCA is a major component of Lewy bodies, inclusions characteristic of sporadic PD. Mouse and human SNCA differ by 7 of 140 amino acids; one difference is at position 53, where an A5T mutation causes PD in humans. In mouse SNCA position 53 is Thr. We have shown that in mice carrying a human A53T SNCA transgene, deletion of mouse SNCA causes earlier onset and higher penetrance of a synucleinopathy than in wild type mice. Thus mouse SNCA protects against the toxicity of human A5T mutant SNCA. We wished to determine which amino acid differences between mouse and human SNCA are responsible for the protective effect of mouse SNCA against human A53T mutant SNCA. We have individually substituted the 6 different human residues into the mouse protein using site-directed mutagenesis, and made stably transfected SH-SY5Y cell lines for each variant as well as human wild type and A5T SNCA and wild type mouse SNCA. Using these cell lines we have shown that 1) differentiation with retinoic acid increases all SNCA levels, 2) SNCA variants form inclusions induced by oxidative stress, 3) protein half-lives are different for each variant, but all increase in differentiated cells. In these assays, G121D and M100L appear to be the 2 variants at the opposite extremes, but no 1 variant more closely resembled human A53T SNCA than wild type mouse SNCA. After analysis of SNCA sequences from 43 species, we decided to make one doubly mutated variant, M100L/G103N. It, along with the other 6 mouse variants, human wild type and A53T SNCA, and wild type mouse SNCA, was tested for toxicity in yeast. Amongst the singly mutated variants, G121D is most toxic, but the M100L/G103N doubly mutated variant is more toxic, indistinguishable from human A53T mutant SNCA and completely inhibiting yeast growth. We are now generating transgenic mice expressing these variants to test their toxicity in a mammalian nervous system.

Development of a genome-wide mutation association study (GMAS) for mutagen-induced modifying alleles. William Dove1, James Amos-Landgraf1, Linda Clipson1, Richard Halberg1, Kathleen Krentz2, Alexandra Shedlovsky1, Michael Newton3, David Adams4. 1) McArdle Lab, Univ Wisconsin, Madison, WI; 2) Departments of Statistics and of Biostatistics and Bioinformatics, Univ Wisconsin, Madison, WI; 3) Experimental Cancer Genetics, Sanger Institute, Hinxton, Cambs., UK. A strategy to scan the entire genome for dosage-sensitive loci at which heterozygosity for a point mutation modifies a phenotype of interest is complementary to genome-wide association studies (GWAS) in the human and the discovery of polymorphic modifying loci among inbred mouse strains. Genetic drift in non-essential or regulatory regions of the genome may bias the spectrum of polymorphisms toward gene deserts.Heterozygosity of inbred experimental murine species eliminates recessive detrimental alleles in essential genes. The strategy we are developing for the Min (Multiple intestinal neoplasia) phenotype of the mouse is called “GMAS” (genome-wide mutation association study). Germline mutagenesis by ENU in the mouse gives forward mutation rates in the order of 10-3 per locus. A kindred can be founded from a member of a 1000-animal first-generation mutational library and quantitatively progeny-tested by generating a cohort of offspring carrying the cancer-predisposing mutation, Min. A kindred can now be reconstituted from cryopreserved sperm, remaining on the C57BL/6J (B6) background. Mapping is essential to connect a candidate modifying allele to the salient segment of the mutagenized genome before targeted re-sequencing. To map a candidate modifying allele without outcrossing and encountering the attendant “noise” of the many polymorphic modifiers of Min, we are developing an “isogenic mapping” panel: six B6-SNP “mapping partners” created by germline ENU mutagenesis followed by extensive inbreeding. Genomic sequencing on the Solexa-Illumina platform to 4X coverage of the first such partner, B6-SNP1, has identified 1026 candidate markers. In a pilot, four of 16 candidate markers have been explicitly confirmed by demonstrating a doublet site in Sanger sequencing of DNA from (B6-SNP1 X B6/F1 progeny sired by bona fide B6/F1 males).
The Mouse Phenome Database (MPD). http://mousemutant.jax.org/. The presentation will enumerate the many advantages of studying spontaneous mutant mice and summarize current resource offerings and metrics.

Phenodeviant Search process, atypical mice are identified from among the millions of inbred mice produced yearly at JAX and isolated. This not only ensures genetic stability within the originating strain, but also provides a pool of potential spontaneous mutant mouse strains for further characterization. After colonies are established, heritability is proven, and the modes of inheritance are determined, subchromosomal locations for each mutant locus are established by backcross or intercross in conjunction with SNP genotyping. In place of Sanger-based sequencing of promising candidate genes from select mutant strains, Illumina GAIIx-based high-throughput sequencing is now being incorporated to broaden the scope of mutation detection and dramatically shorten the time to causative gene identification. A general phenotypic assessment, cryopreservation of embryos or gametes, and archiving mutant DNA completes the characterization. Findings for each mutant strain are disseminated through the scientific literature or on the MMR website at http://mousemutant.jax.org/. The presentation will enumerate the many advantages of studying spontaneous mutant mice and summarize current resource offerings and metrics.

New tools and features will be demonstrated.

The Wellcome Trust Sanger Institute Mouse Genetics Project (Sanger MGP) is a European Mutant Mouse Archive (EMMA) partner and deposits archived mouse lines generated by the project at EMMA distribution centres for a sustainable resource to the scientific community. Mouse lines are also sent to the KOMP repository for archiving and distribution. The mutant mouse lines are generated from the EUCOMM and KOMP ES cell resource produced at the Sanger Institute. These mouse lines are characterised through primary phenotypic studies and both the data (Sanger Mouse Resource Portal; Europhenome) and mouse resource (IKMC; EMMA; KOMP Repository) are made available to the scientific community. Demand on the archiving of the mouse models has significantly increased over the past 2 years as the number of mutant mouse lines has increased.

Development of sperm freezing and IVF techniques for the C57BL/6N strain is envisaged to compliment the embryo cryopreservation and allow more mouse lines to be cryopreserved more efficiently and economically. Prior to archiving at a distribution centre and whilst being actively maintained on the shelf for phenotypic characterisation we also offer the potential early opportunity to source the mice should they be available beyond our phenotyping requirements. As well as supporting the international academic scientific community our resource has supplied more detailed secondary phenotyping studies to consortia such as Genome Canada and EUMODIC partners. The scientific community has shown significant interest in our mouse lines and Sanger MGP now contributes ~25% of the EMMA exports. By the end of 2010 we had completed more than 500 international exports of live mice and embryos to the scientific community and repositories.


The Mouse Phenome Database (MPFD; phenotype.jax.org), maintained at The Jackson Laboratory, is the product of an international community effort to collect quantitative phenotypic and genotypic data on laboratory mouse strains. Since last year, MPFD added several comprehensive datasets on a broad range of phenotypic domains, including exercise-induced neurogenesis, dietary restriction on lifespan, immune response to pathogens, airway resistance, pain-related behavioral characteristics, autism-relevant behaviors, exploratory behavior, toxicogenetic analysis, reproductive parameters, and many aging-related phenotypes. MPFD is also in the process of integrating gene expression datasets, which are eligible for analysis with MPFD tools and correlating with phenotypic data. In addition, MPFD is now providing assistance for association analysis (EMMA) in conjunction with collaborators at UCLA (E. Eskin Lab). MPFD is indispensable for helping researchers select optimal strains for many research applications. MPFD contains: •Data for over 600 strains of mice (inbred, RI, congenic, etc.) •Hundreds of baseline measurements of biomedically-relevant phenotypes •A growing collection of data from treated mice, e.g., drugs; carcinogenic or toxic compounds; high-fat diet •Detailed protocols and environmental conditions of the test animals •New SNP datasets (more strains and more genomic locations) •Gene expression microarray data •Pathology survey of aged inbred strains of mice. New tools and features will be demonstrated.


The Wellcome Trust Sanger Institute Mouse Genetics Project (Sanger MGP) is a European Mutant Mouse Archive (EMMA) partner and deposits archived mouse lines generated by the project at EMMA distribution centres for a sustainable resource to the scientific community. Mouse lines are also sent to the KOMP repository for archiving and distribution. The mutant mouse lines are generated from the EUCOMM and KOMP ES cell resource produced at the Sanger Institute. These mouse lines are characterised through primary phenotypic studies and both the data (Sanger Mouse Resource Portal; Europhenome) and mouse resource (IKMC; EMMA; KOMP Repository) are made available to the scientific community. Demand on the archiving of the mouse models has significantly increased over the past 2 years as the number of mutant mouse lines has increased.

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POSTER: Resources and databases

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

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LAMHDI, the Linking Animal Models to Human Disease Initiative. Janan T. Eppig1, Dave Anderson12, Anita Bandrowski, Bobby-Jo Breitkreutz, Brian Canada11, Andrew Chatr-aryamontri1, Keith Cheng1, P. Michael Conn1, Kara Dolinsky3, Mark Ellision1, Jeffrey S. Grethe3, Melissa Haendel1, Joseph Keminji13, Stephen Larson1, Maryann Martone1, Chris Mungall1, Carlo Tornai1, Olga Troyanskaya1, Mike Tyers1, Monte Westerfield1. 1) Jackson Laboratory, Bar Harbor, ME; 2) Lawrence Berkeley National Laboratory, Berkeley, CA; 3) Oregon Health & Science Univ., Portland, OR; 4) Penn State, College of Medicine, Hershey, PA; 5) Princeton Univ., Princeton, NJ; 6) Samuel Lunenfield Research Institute, Univ. of Toronto, Toronto, Ontario, Canada; 7) UCDSD Dept. of Neuroscience, San Diego, CA; 8) UCSD National Center for Microscopy and Imaging Research, San Diego, CA; 9) Univ. of Edinburgh, Edinburgh, Scotland; 10) Univ. of Oregon, Institute of Neuroscience, Eugene, OR; 11) Univ. of North Carolina, Columbia, SC; 12) Washington National Primate Research Center, Seattle, WA; 1; 13) Wisconsin National Primate Research Center, Madison, WI.

We have developed LAMHDI (www.lamhdi.org), a free Web-based resource to help researchers identify and locate the most appropriate human disease models across species (mice, rats, zebrafish, flies, and yeast, with others in the pipeline). LAMHDI builds on genetic linkages between models, using homologous genes (principally from MGD) to span humans and animal models. This project leverages public web resources like MGD, which shares the aims of increasing access to a wide range of human disease models. We use gene orthology and pathways as key linkages; new work includes matching specific phenotypes and common pathways. The goal is to make better use of existing model organisms and data about them, and provide the ability to discover relationships between diseases, phenotypes, and genes to further our understanding of disease. This work is supported by contract HHSN268200800014C from NIH/NCRR.

232B

Novel mutation discovery on ENU mouse mutagenesis by exome targeted re-sequencing. Ryutaro Fukumura, Hayato Kotaki, Takuya Murata, Shigeru Makino, Yuji Nakai, Yuchi Ishitsuka, Yoichi Gondo. RIKEN BRC, Japan.

We developed a reverse genetics tool to provide allelic series of point mutation in any mouse genes. We have produced about 10,000 Generation-1 (G1) male mice derived from ENU-treated C57BL/6J males. We have preserved them as frozen sperm and extracted each genomic DNA for the mutation screening of target genes. Thus, the dual archives of them are considered to be the Mutant Mouse Library (MML). By the direct Sanger sequencing analysis, the mutation rate of MML was estimated to be about 1 mutation/Mbp in a G1 genomic DNA; namely, each G1 mouse has about 3,000 mutations in the 3,000 Mbp of the genome. Therefore, a total of 30,000,000 mutations are reserved in the MML. To significantly enhance the mutation detection efficiency, we have started to re-sequence the G1 mouse genomic DNAs in the library by the next generation sequencer (NGS). Our final goal is to detect all the ENU induced mutations in the G1 genome; however, we decided to firstly find the mutations by targeting all the protein coding sequences in the mouse genome, considering the throughput and cost performance. We have so far targeted all exons (about 49.6Mbp) for four G1 genomes. Two G1 genomes were in DBF1 (DBA2/J x C57BL/6J) and the other were C57BL/6J genetic background. By using AB SOLiD sequencer, we covered about 85% of the targeted sequence were covered by at least 10-fold read depth. The average read depth was 46 folds in the 49.6Mbp targeted sequences. We have so far found 61, 34, 65 and 54 novel mutations from each G1 genome. We have estimated the mutation detection efficiency with known 1000 SNPs from the two exons from the DBF1 genetic background as positive controls. The known SNPs were consistently detected with >90% efficiency where the read depth is more than 10 folds.

233C

Associating phenotype with genotype in 17 sequenced mouse genomes. Leo Goodstadt1, Thomas M. Keane2, Peter Danek2, Richard Mot1, David J. Adams2, Jonathan Flint1. 1) The Wellcome Trust Centre for Human Genetics, University of Oxford, UK; 2) The Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

We have sequenced the genomic sequence for 17 strains of laboratory and wild derived mice and identified 129.3M Single Nucleotide Polymorphisms (SNPs) and 20.5M short indels (<10 bp). Using 17.5Mb of finished-quality sequence from a non-reference strain, we obtained independent estimates of the false positive and false negative rates. This also allowed us to survey the amount of sequence variation in genomic regions inaccessible to current generation sequencing technology. For example, in the divergent, wild-derived SPRET/EJ mouse, 23.3% of whose genome is inaccssible, we estimate that 46% of SNPs remain to be discovered. We were interested in using the sequence of these mouse strains to understand the genomic architecture of variants underlying complex traits. Candidate functional variants could be identified in 718 out of 897 QTLs mapped in > 2,000 heterogeneous stock mice derived from eight of the sequenced strains. The median number of variants at each QTL was 7, while 10% of QTLs had only a single candidate functional variant. We found that candidate functional variants contributing to large effect QTLs were over-represented in genic regions, particularly introns, while the converse was true for small effect sizes. In the 3% of QTLs with the strongest effect size (exceeding more than 10% of phenotypic variance), we found significant enrichments for coding variants. However, these were more likely to arise from indels and structural size than SNPs.

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EMMA - European Mouse Mutant Archive. Michael Hauge1,2, Giusso Tochcini-Valentini3, Steve Brown1, Urban Lendahl2, Yonecylene Demengeot4, Ewan Birney5, Ramiro Ramirez-Solis5, Yann Heraud5, Luis Montoliu6, George Kollas6, Thomas Rulicke7, Radislav Sedlacek8, Raaja Soininen9, Martin Hrabé de Angelis10. 1) Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Germany; 2) The EMMA Network, Monterotondo Scalo, Italy.

The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archival service for its mouse mutant lines and access to a wide range of disease models for pre-clinical research tools. A full description of the project and a list of those services can be viewed on the EMMA website at http://www.mammareport.org. The EMMA network is comprised of ten partners who operate as the primary mouse repository in Europe and is funded by the participating institutes and the European Commission FP7 Capacities Specific Program. EMMA’s primary objectives are to establish and manage a unified repository for maintaining mouse mutations and to make them available to the scientific community. In addition to these core services, the consortium can generate germ-free (axenic) mice for its customers and also hosts courses in cryopreservation. All applications for archiving and requests for mutant mouse strains are submitted through the EMMA website. Mouse strains submitted for archiving are evaluated by EMMA’s external scientific committee. Once approval has been granted depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or spermatozoon cryopreservation. Strains held under the EMMA umbrella can be provided as frozen materials or re-derived and shipped as live mice depending on the customer’s needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery. All animals supplied by EMMA are classified as SPF in accordance with the FELASA recommendations. EMMA is a founding member of FIMRe (International Federation of Mouse Resources) and actively cooperates with other leading repositories like TJL and the MMRRC in the US and BRC RIKEN from Japan.

235B

Sequencing of the mouse pseudoautosomal region. Takaaki Kasahara1, Kuniya Abe1, Tadafumi Kato1. 1) RIKEN Brain Science Institute, Wako-shi, Saitama, Japan; 2) RIKEN BioResource Center, Tsukuba-shi, Ibaraki, Japan.

Mouse pseudoautosomal region (PAR) is an enigmatic region of the genome. The current genome assembly of the mouse does not contain the sequence of PAR. PAR, a narrow region of homology between the termini of X and Y chromosomes, mediates proper sex chromosome pairing and segregation. Since meiotic recombination occurs very frequently in the PAR during spermatogenesis, the mutation rate is supposed to be extremely high especially in the mouse PAR that seems shorter than other PARs such as in humans. Therefore the mouse PAR tells us the genome evolution driven by the recombination. Recently we identified the mouse gene encoding melanin synthesizing enzyme (Hmmt) located on the PAR (Kasahara et al., PNAS 107, 6412-6417 (2010)), and we isolated a BAC clone containing almost the entire sequence of mouse PAR. In this study, we sequenced the BAC clone with a combination of Sanger method (primer extension method and sequencing of the plasmid shotgun library and deletion constructs) and Roche 454 sequencing. We identified four genes located on the PAR. One was Hmmt, another Xo which was genetically expected to be on the PAR, another Xgay, and the other one was a novel gene. The four genes shared three characteristics of nucleic acid sequence: high GC content in exons, shortened introns, and high-density mnisatellites in introns; and repetitive sequences comprised most of the intergenic regions. The mouse PAR is the second sequenced PAR after the human PAR1. There is considerable conservation of synteny between mouse PAR and human PAR1. The interspecies comparison of PAR will enable us to determine the evolution and future of mammalian PAR.
236C

**Construction of reciprocal chromosome substitution strains from 129P3/1 and C57BL/6JbSix mice.** Cailin Lin, Natalia Bosak, Theodore Nelson, Maria Theodorides, Zakiyah Smith, Matthew Kirkey, Mauricio Avigdor, Brian Gantick, Amin Khoshnevisan, Anna Lysenko, Danielle Reed, Alexander Bachmanov. Monell Chemical Senses Center, Philadelphia, PA.

The 129P3/J (129) and C57BL/6JbSix (B6) inbred strains differ in taste responses, ingestive behavior, alcohol consumption, body size and adiposity. Genome scans of crosses between these strains have detected QTLS for these phenotypes that cluster on chromosomes (Chr) 1, 2, 7, and 9 (Ad2p2, Adp1, Adp5, 4p7q, B6p6, B6p9, Natt1, Natt2, Scaq1). Chromosome substitution strains (CSSs) are a useful resource for positional identification of these QTLS, but extant B6 and 129 CSSs involve different substrains (129sv/129M) and which are genetically and phenotypically distinct from the substrains used in our studies. We therefore initiated construction of reciprocal CSSs for Chr 1, 2, 7, and 9 from the 129P3/J and C57BL/6JbSix strains using a ‘speed genomics’ approach. During the first two backcross generations (N2 and N3) a genome scan was conducted to identify optimal breeding partners. In subsequent backcrosses, donor chromosomes were genotyped to prevent the loss of fragments due to double-crossovers. To ensure that the QTLS were retained, we phenotyped several incipient strains and conducted linkage analyses; these analyses confirmed the original QTLS on Chr 1 (Natt1), Chr 2 (B6p9, Adp5), Chr 7 (4p7q, Adp5) and Chr 9 (Natt2, B6p6, Adp5, Scaq1). Some CSS strains are now complete (C57B6/JbSix-Chr 7129P3/J C57B6/JbSix-Chr 9129P3/J) and others are nearing completion. Supported by NIH grants R01DC000882, R01AA10128 (A.A.B), R01DK058797 (D.R.R.) and the Center for Inherited Disease Research (CIDR).

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**The Knockout Mouse Project (KOMP) Repository.** Kent Lloyd1, Pieter de Jong2, Ray O'Neil1. 1) Mouse Biology Program, University of California Davis, Davis, CA; 2) Children's Hospital Oakland Research Institute, Oakland, CA; 3) National Center for Research Resources (NCRR)/National Institutes of Health (NIH), Bethesda, MD.

The KOMP Repository is the sole archive and distribution repository for all products (vectors, targeted ES cells, mice, and germplasm) generated in the NIH KOMP Mutagenesis Program conducted by the CSD consortium (CHORI, The Sanger Institute, and Regenomer). The Repository ensures the quality, preservation, protection, availability, and accessibility of KOMP products to the research community. Inaugurated in June 2007, the Repository is built upon the expertise of technical resources, facilities, and a large archive of available mouse strains. It provides a source of historical data to assist researchers in finding information on a particular gene even though the symbol might have changed. MGI tracks all gene symbol changes and provides automatic notices when products become available, access customer and technical service (1-888-KOMP-MICE, service@komp.org), read news, follow the KOMP blog, view FAQs, download protocols, receive rebates using the KOMP Sharing Plan, and stay connected through the KOMP Repository mobile “app” (m.komp.org). Products and services are available to for a reasonable fee. Of the more than 5500 targeted genes available, more than 4000 products (ES cells, line mice, cell lines) have been ordered and distributed to over 1200 investigators. More than 4000 users registered at komp.org have expressed interest in over 3000 genes. Average germline transmission rate is ~65% per clone. Eventually, products for 8,500 knockout targeted genes, most conditionally ready, will be available.

238B

**Optimization of RNA-Seq to Capture the Transcriptome of a Single Blastocyst.** Chiao-Ling Lo1,2, Hyunho Chun2, Amy C. Lossie1,2. 1) Department of Animal Sciences, Purdue University, West Lafayette, IN; 2) Purdue Interdisciplinary Life Science Program (PULSe), Purdue University, West Lafayette, IN; 3) Department of Statistics, Purdue University, West Lafayette, IN.

Transcriptome studies capture a molecular snapshot of the complement of transcripts and produce a molecular signature that provides clues to the functional identity of the cell, tissue or organism. With the powerful tool of next generation sequencing techniques, researchers can use RNA-Seq to analyze the transcriptome at single-base resolution. However, RNA-Seq requires ~200 ng of RNA-depleted mRNA, which often necessitates pooling of small biological samples, thereby increasing within sample variability. For studies aimed at identifying transcriptome changes during pre-implantation development, pooling is discouraged because the expression changes are often small and are hard to distinguish if variability is high. To overcome this obstacle, we are optimizing methods to perform RNA-Seq in single mouse blastocysts. Our long-term goal is to delineate the dynamics of global gene expression profiles throughout pre-implantation development, and identify genes that are critical to these processes. Our protocol is a variation of Tang et al. 2010 Nature Protocols, and is optimized for use on the ABI SOLID platform. Specifically, we include steps for genotyping embryos, quantifying input material, cDNA amplification and multiplex library preparation. We are able to yield more than 1 µg of purified cDNA from a single blastocyst and construct deep-sequencing libraries that are ready for emulsion PCR. We envision our RNA-Seq protocol to facilitate the determination of transcriptome studies on small biological samples and ultimately enhance our understanding of the dynamics of gene expression changes during pre-implantation development.

239C

**An ENU-induced mutation in mouse Sufu caused misregulation of the repressor form of the GLI3 transcription factor.** Shigeru Makino, Ryutaro Fukumura, Takuya Murata, Yuichi Ishitsuka, Hayato Kotaki, Yoichi Gondo. RIKEN BioResource Center, Tsukuba, Ibaraki, Japan.

Hedgehog (Hh) signaling plays important roles in development and cancer. Genetic analysis showed that SUFU is an indispensable negative regulator in the Hh signal from a membrane receptor to Sonic hedgehog (Shh) to GLI transcription factors. Recent reports have suggested three important roles of SUFU functions, although the detailed mechanism is yet unknown; Role 1: SUFU for the proteasome dependent proteolytic processing of -GLI3REP to the repressor form GLI3REP, and Role 3: SUFU has roles for the regulation of neither GLI3FULL or -GLI3REP activity nor stability.

To understand the function of Sufu in vivo, we established Sufu mutant mice by the RIKEN ENU-based gene-driven mutagenesis system. One of them, SufuT396I, is a missense mutation and shows recessive embryonic lethality at ~E15. T396I embryos were strikingly similar to the mutant mice by the RIKEN ENU-based gene-driven mutagenesis system. One of them, SufuT396I, is a missense mutation and shows recessive embryonic lethality at ~E15. T396I embryos were strikingly similar to the GLI3 mutant including open brain and severe polydactyly, implying misregulation of the repressor form of GLI3 protein. Thus we analyzed the expression of the GLI proteins in T396I embryos and found that the -GLI3REP protein was reduced but -GLI2FULL and -GLI3FULL were intact. This suggested that the Thr396 residue of SUFU may have specific function for the -GLI3REP expression. The stability studies of -GLI3REP in cultured cells showed that the T396I mutant protein had stabilized -GLI3REP less than the wild type. Furthermore, we found that the wild-type SUFU, but not T396I, had an ability to tether the GLI3 protein, which localized to the nucleus without SUFU, in the cytoplasm. These results suggested a novel regulatory mechanism that SUFU might tether the GLI3REP protein in the cytoplasm to prevent from degradation depending on the Thr396 residue. Our findings are consistent with the above-mentioned Roles 1 and 2 but not with 3.

240A

**The shifting sands of gene nomenclature: New data, new symbols.** Lois J. Maltais, Judith A. Blake, Janan T. Eppig, Dept Bioinformatics, Jackson Laboratory, Bar Harbor, ME.

“The world hates change yet it is the only thing that has brought progress.” -Charles Kettering. Assigning symbols and names to genes is based on 1) available data, including published papers, communication with authors and gene family specialist advisors and 2) sequence and comparative databases, such as Entrez-Gene, Homologene, Pfam, Ensembl, and Uniprot. Despite efforts to ensure symbols and names are appropriate, newly emerging data could indicate the nomenclature is misleading and inadequate. In these instances, change to the existing nomenclature must occur to reflect current knowledge. Over the years many symbols and names have changed several times. Yet, people are resistant to change, one of their concerns being whether change will result in confusion. Fortunately, the Mouse Genome Informatics (MGI) resource (www.informatics.jax.org), is a valuable source of historical data to assist researchers in finding information on a particular gene even though the symbol might have changed. MGI tracks all gene symbols and previous ‘official’ symbols and unofficial literature symbols are retained as synonyms. The nomenclature history of symbol and name changes to a gene with supporting references is available for the user. In addition, MGI maintains an internal nomenclature database containing communications and reasons for symbol assignments and/or changes should anyone question why a symbol was assigned or who was involved in the nomenclature process The MGI nomenclature group, the Rat Genome Database (RGD) nomenclature group (www.rgd.mcw.edu/), and the HUGO Gene Nomenclature Committee (HGNC, www.gene.ucl.ac.uk/nomenclature/index.html) work collaboratively in assigning official nomenclature and ensuring it remains current. This joint effort contributes to resolving nomenclature issues for the scientific community. If nomenclature changes are deemed necessary, the orthologous gene in all three species will be dealt with simultaneously. As Alfred North Whitehead said, “The art of progress is to preserve order amid change and to preserve order amid change.”
Integrating “indexing” and curation at MGI to functionally annotate developmental biology. Monica McAndrews-Hill, Carol Bolt, Jim Kadin, Joel Richardson, Martin Ringwald, Janan Eppig, Judith Blake, MGI Team. Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, ME. Mouse Genome Informatics (MGI) is the premier model organism database for the laboratory mouse. MGI (www.informatics.jax.org) includes multiple databases, such as the Mouse Genome Database (MGD) and Gene Expression Database (GXD). It is the authoritative source for nomenclature, alleles, and genome sequence features. It combines developmental expression, functional and phenotypic data to put molecular genes in the context of developmental biology. Data are obtained and integrated via manual curation of the biomedical literature, direct contributions from individual investigators and downloads from bioinformatics resource centers. Currently, MGI contains 12,742 genes with gene traps, 193,759 expression images and 33,895 mouse genes with GO annotations. In addition to managing large data loads, curators survey 149 journals monthly, adding over 12,000 articles to the database in 2010. Literature curation efforts begin by identifying publications containing mouse developmental expression, functional and phenotypic data using the literature management tool Quoqa (www.quoqa.com) to access and search PubMed. Through the “indexing” process, articles are associated with genes as the next step in curation. MGI employs the text-mining tool ProMiner (www.scai.fraunhofer.de/en/products.html) to automate this. ProMiner matches text in each article to a list of keywords then highlights mouse and human gene symbols, names and synonyms. After indexing to the correct gene or genes, the article enters a queue for annotation. Expert curators add data for the proper genes to the database. Data are summarized and presented on a marker detail page for each gene. Users can find information about chromosomal location, sequences, alleles and phenotypes, gene ontology classifications, developmental expression, external links and original references. This poster will illustrate specific examples of developmental data supported in MGI. Supported by NIH grants HG000330, HG002273, HD062499

NIH-funded whole exome resequencing of mouse monogenic mutants and quantitative trait loci. Jennifer L. Moran1, Evan Mauceli2, Snearav Sigurdsson3, Tim Fennell4, Lauren Ambrogio1, Miriam H. Meisler1, John C. Schimenti3, Jane Wilkinson1, Stacey Gabriell1, David R. Beier4, Federica di Palma1, Kerstin Lindblad-Toh1. 1) Broad Institute, Cambridge, MA; 2) Dept of Human Genetics, Univ of Michigan, Ann Arbor, MI; 3) Center for Vertebrate Genomics, Cornell University, Ithaca, NY; 4) Genetics Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA. To fill this need, The Jackson Laboratory (JAX) has committed to increasing the number of Cre lines available to the scientific community. The JAX Cre Repository currently houses and distributes the single largest collection of Cre driver strains totaling more than 200 lines, including 150 that are currently distributed as live colonies. The JAX Cre Repository has embarked on an ambitious project to add value to these strains by comprehensively characterizing Repository Cre lines. In collaboration with the Neuroscience Capitalizing on mouse gene targeting projects of the International Knockout Mouse Consortium (IKMC) will require that a large, diverse set of well-characterized Cre driver lines. To this end, The Jackson Laboratory (JAX) has committed to increasing the number of Cre lines available to the scientific community. The JAX Cre Repository currently houses and distributes the single largest collection of Cre driver strains totaling more than 200 lines, including 150 that are currently distributed as live colonies. The JAX Cre Repository has embarked on an ambitious project to add value to these strains by comprehensively characterizing Repository Cre lines. In collaboration with the Neuroscience

The JAX Cre Repository: Improving the utility of Cre driver strains. Stephen A. Murray, Caleb Heffner, C. Herb Pratt, Michael Saez, Cathleen Lutz, Brandon Grossman, Yashoda Sharma, Leah Rae Donahue. The Jackson Laboratory, Bar Harbor, ME. The JAX Cre Repository: Improving the utility of Cre driver strains. Stephen A. Murray, Caleb Heffner, C. Herb Pratt, Michael Saez, Cathleen Lutz, Brandon Grossman, Yashoda Sharma, Leah Rae Donahue. The Jackson Laboratory, Bar Harbor, ME. Capitalizing on mouse gene targeting projects of the International Knockout Mouse Consortium (IKMC) will require that a large, diverse set of well-characterized Cre driver lines. To this end, The Jackson Laboratory (JAX) has committed to increasing the number of Cre lines available to the scientific community. The JAX Cre Repository currently houses and distributes the single largest collection of Cre driver strains totaling more than 200 lines, including 150 that are currently distributed as live colonies. The JAX Cre Repository has embarked on an ambitious project to add value to these strains by comprehensively characterizing Repository Cre lines. In collaboration with the Neuroscience

The FaceBase Cre driver project: generating new tools for orofacial clefting research. Stephen A. Murray, Jocelyn Sharp, Caleb Heffner, John Flaherty, Thomas Gridley, Leah Rae Donahue. The Jackson Laboratory, Bar Harbor, ME. Orofacial clefting is one of the most common birth defects in humans, affecting approximately 1 in 700 live births. This frequency highlights the complexity of craniofacial morphogenesis, which requires precise regulation of gene expression changes, alterations in cell physiology and morphogenetic movements. The mouse has played an instrumental role in advancing our understanding of the mechanisms that govern mid-face and palate development. Future progress, however, will require an increasingly sophisticated set of genetic models and tools. As part of the NICHD-funded FaceBase Consortium, we will generate 15 Cre driver strains to facilitate orofacial clefting research in the mouse. These driver strains will comprise both inducible and non-inducible approaches, and include a fluorescent marker for simultaneous visualization of Cre expression patterns. The individual driver promoters have been chosen to encompass both early midface and primary/secondary palate development and fusion. These tools are designed to complement existing lines, allowing users to define gene function in new cell types and regions. A combination of approaches will be employed to produce the lines, including BAC transgenic, knock-in and targeting to the ROSA26 locus. In addition to detailed characterization of Cre functionality in the midface and palate, we will use our existing comprehensive characterization pipeline to ensure specificity and carefully define any non-target activity. These strains will include, Krt6a-Cre, deltaNp63-Cre, Tbx22-Cre and Lhx8-Cre. All of the data will be made available as part of the FaceBase effort, and lines will be redistributed through the JAX FaceBase Repository, which includes both Cre lines and other mouse models and tools useful in orofacial clefting research. Development and rapid distribution of these lines will greatly enhance the genetic “toolbox” available to the scientific community for understanding the mechanisms that govern orofacial clefting.
associated annotations related to the eye. The rest represent novel observations either because the mutant mice have not been generated and studied, or because the previously known phenotypes do not include the eye. The full exploitation of this valuable resource depends on access to a comprehensive functional and molecular characterization of mouse lines and to sufficient capacities for archiving and distributing to the biomedical research community. THE CHALLENGE - Europe has built a strong research base for mouse functional genomics, by establishing world-leading facilities for high-throughput first-line phenotyping (European mouse clinics), archiving and distribution (organized in EMMA - the European Mouse Mutant Archive), and by defining cross-laboratory standards for mouse phenotyping, archiving and distribution. However, the existing capacities do not match the increasing demand by the community; moreover, sustainable funding solutions are not always in place. This endangers Europe’s strong position in this important research area. THE SOLUTION - Infrafrontier is establishing a research infrastructure for the systemic phenotyping, archiving and distribution of mouse models of human diseases. Infrafrontier builds the required capacities for systemic phenotyping, archiving and distribution of mouse models, based on a sustainable funding concept. Infrafrontier promotes common pan-European standards for mouse phenotyping, archiving and distribution and the development of novel technologies in these areas. The pan-European Infrafrontier Research Infrastructure will provide open access for individual researchers and for large-scale collaborative efforts such as the International Mouse Phenotyping Consortium (IMPC). Infrafrontier has been prioritized in the ESFRI Roadmap Report and is supported by the European Commission. www.infrafrontier.eu.

The Mouse Phenotyping Core "A Small Animal Imaging Facility". Corey L. Reynolds, Julie A. Santosuosso. Office of Research, Baylor College of Medicine, Houston, TX. The Mouse Phenotyping Core (MPC) is a comprehensive, state of the art small animal imaging facility located at the Baylor College of Medicine. The MPC is located within an AAALAC accredited animal facility on the main Baylor campus and is classified as a satellite facility capable of housing animals for studies. The facility offers non-invasive testing including Non-Invasive Blood Pressure, Bone Densitometry, Metabolic cages, Plethysmography, ECG/Gene, running wheels and treadmills. There are also several pieces of high-end imaging equipment including Ultrasound, X-ray/Fluorescence imager, CT/SPECT and MRI. There is a dedicated surgical suite in which we offer several surgical services such as aortic banding, telemetry device implantation, and osmotic pump implantation. Data analysis for all testing performed is also available as a service or can be taught to core users. The core works with investigators from all over the country to design protocols to fit their experimental design and strives to stay abreast with the most current technologies. Facilities such as the MPC are important to research because they allow the number of animals used to be decreased and the financial burdens placed on laboratories to be minimized.

High-throughput exploration of the genotype to discover genes important for the mammalian eye. Ramiro Ramirez-Solis, Ed Ryder, Richard Houghton, Joanna Bottomley, Jacqui White, Mouse Genetics Project, Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom. The Sanger Mouse Genetics Project is a large scale, high-throughput mouse production and primary phenotyping exploration of the mouse genome to reveal genotype-phenotype relationships at the organismal level in a broad set of phenotypic areas of biomedical importance. To date, mutant lines for over 700 different loci have been generated and nearly 300 have undergone a primary phenotyping revealing a rich set of confirmatory and novel genotype-phenotype relationships. A significant proportion of genes for the MGP have been selected based on hypothesis driven by the community at large, as well as from collaborations internal and external to the Sanger. The rest of the mutant genes have been selected based on the absence of previously known phenotypic information for mutant mice. This set of mutants has revealed an enormous amount of novel information with the potential to open several completely new research areas. The set of phenotypic tests is continually being optimized by eliminating tests that do not provide information or by adding new tests that enrich the screen. We will present the global characteristics of the phenotypes discovered, as well as some individual highlights in diverse areas of biomedical importance. The mutant strains and the phenotypic data are freely available to the scientific community to facilitate further research. Mutant strains can be obtained directly from the Sanger Institute or from the EMMA and KOMP repositories. The phenotypic data is available through the Europhenome or Sanger Mouse Portal (www.sanger.ac.uk/mouse portal) websites. The Mouse Phenotyping Core is a comprehensive, state of the art small animal imaging facility located at the Baylor College of Medicine. The MPC is located within an AAALAC accredited animal facility on the main Baylor campus and is classified as a satellite facility capable of housing animals for studies. The facility offers non-invasive testing including Non-Invasive Blood Pressure, Bone Densitometry, Metabolic cages, Plethysmography, ECG/Gene, running wheels and treadmills. There are also several pieces of high-end imaging equipment including Ultrasound, X-ray/Fluorescence imager, CT/SPECT and MRI. There is a dedicated surgical suite in which we offer several surgical services such as aortic banding, telemetry device implantation, and osmotic pump implantation. Data analysis for all testing performed is also available as a service or can be taught to core users. The core works with investigators from all over the country to design protocols to fit their experimental design and strives to stay abreast with the most current technologies. Facilities such as the MPC are important to research because they allow the number of animals used to be decreased and the financial burdens placed on laboratories to be minimized.
POSTER: Resources and databases

The Mouse Regional Resource Centers are national consortia that maintain and distributes scientifically valuable, genetically engineered mouse strains and ES cell lines. In operation since 2000, the MMRRC national network consists of 4 distribution facilities and an information coordinating center. Several large collections are available from the MMRRC. Among the collections offered are the Pleiades Promoter Project lines, a subset of the NIH Neuroscience Blueprint cre-expressing mice, the Gene Expression Nervous System Atlas (GENSAT) project mice and a Sanger Institute gene trap ES cell set. In addition to large collections, a wide variety of mutants have been accepted from individual investigators. Recent additions include a KO of the ifnar1 gene. These mice lack type I IFN receptor functionality and exhibit increased susceptibility to viral infection, elevated levels of myeloid lineage cells and a reduced immune response to immunostimulatory DNA. Many useful floxed alleles are also available (e.g., B6.Cg-Tg(m2Bbk/Mmjax)). Numerous tool strains are offered, such as the DEREG transgenic line (depletion of regulatory T cells) which expresses a BAC transgene consisting of a simian diptheria toxin receptor-enhanced gfp (DTR-eGFP) fusion protein under control of the Foxp3 promoter. This strain allows the detection or DT-induced ablation of CD25+CD4+ T cells. Another useful line is the ‘RCE: dual reporter’ mouse. This harbors a knock-in mutation at the endogenous Gt(ROSA)26Sor locus. Expression of EGFP is prevented by two upstream STOP cassettes. After removal of the flanked STOP cassettes via cre- and Flp-mediated recombination, the EGFP reporter is expressed in tissues where the expression patterns of the individual promoters driving Cre recombinase and Flp recombinase overlap. An on-line resource provides information related to mutant strains (http://www.mmrrc.org). Strain records retrieved from the MMRRC database include phenotype descriptions, a listing of supporting references and links to related online resources. The MMRRC is supported by the NCCR/NIH.

25A

High-throughput genotyping, quality control and gene expression analysis of targeted mutations in the mouse. Ed Ryder, Diane Gleeson, Debarati Bhattacharjee, Ross Cook, Kalpesh Jhaiveri, Matthew Hardy, Claire Haskins, Sapna Vyas, Ramiro Ramirez-Solis, Mouse Genetics Project. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

The Sanger Institute is an active member of the international EUCOMM and KOMP consortia, whose goal is to produce conditional knockouts in ES cell lines and mice as a resource for the research community. As part of these projects and the EUROMIC European phenotyping collaboration, the Mouse Genetics Project (MGP) is involved in the generation of mouse mutants from micro-injection of ES cells to primary phenotyped mice. In addition to high throughput genotyping, quality control methods are discussed for confirming the targeting and mutant allele mouse colonies prior to release to the phenotyping pipelines, external laboratories and the EMMA and KOMP repositories. We are currently evaluating the level of reduction in the success rate of alleles, which relies on a strong knock-out allele, in a clone, which prevents functional mRNA from being produced (and to allow LacZ expression analysis). The study will determine whether the activity of the splice acceptor and subsequent reduction in gene expression is influenced in an allele-specific manner at all, and if so what percentage of lines could possibly be affected. Approximately 50 genes have been chosen based primarily on sites of expression and availability of material, and include promoterless and promoter-driven lines. This study will give a good indication of the utility of the knockout-first allele in current and future large scale mouse production/phenotyping screens.

25B

Updating the Mouse Reference Genome Assembly. Valerie A. Schneider, on behalf of the Genome Reference Consortium and the NCBI Genome Annotation Team. NCBI, Bethesda MD; The Genome Center at Washington University, St Louis MO; EBI, Hinxton, UK and The Wellcome Trust Sanger Institute, Hinxton, UK.

The current mouse reference genome, MGSCv37, was released in 2007. It is among the highest quality mammalian assemblies, with less than 1% consisting of gaps and more than 96% comprised of finished, clone-based sequence. This assembly, representing the C57BL/6J strain, has facilitated advances in mouse biology, and enabled comparative analyses of the mouse with other species. Recently, this reference has played an important role as a scaffold used to guide the assembly of short-read sequences from other mouse strains. However, genomic analyses enabled by the reference assembly have also revealed deficits within it. Sequences that resist cloning in large insert vectors remain as gaps, while complex, repetitive regions have resulted in localized mis-assemblies. Furthermore, due to inter-strain variation, some genomic regions are insufficiently represented by the single strand of the reference. The Genome Reference Consortium (GRC) assumed responsibility for the mouse reference genome in 2007, and is addressing all of these issues. The GRC welcomes feedback on the assembly and displays information about regions under review on its website (http://genomereference.org). A public release of an updated mouse assembly is planned for late 2011. We will present examples of changes in the upcoming release. NCBI provides bioinformatic support for the GRC and the annotates the mouse assembly as part of its genome annotation pipeline. Features include genes, RefSeq transcripts, placements for genomic, MICEr and gene trap clones, repeats and genomic sequences not included in the assembly. NCBI also provides annotation for several partial assemblies of clone-based sequences from other mouse strains. These annotations can be viewed in the NCBI MapViewer, which permits the simultaneous display of genomic maps with different coordinate systems. We will present data from the recent NCBI re-annotation of MGSCv37 and discuss annotations that will be made on the new assembly.

25C

Clone DB: an integrated NCBI resource for clone-associated data. Valerie A. Schneider, Nathan Bouk, Hsiu-Chuan Chen, Cliff Clausen, Nora Husain, Peter Merc, Lukas Wagner, Donna Maglott, Deanna M. Church. NCBI, NLM, NIH, Bethesda, MD.

The mouse genome sequence for many organisms, including mouse, has transformed the way researchers approach biological questions. However, there is still a need to associate genomic sequence with physical reagents that can be used to perform experiments. We have developed a resource to facilitate this association for one important reagent: clones. Genomic clones form the basis of the mouse reference assembly, while gene trap and gene target clones have been cloned in large insert vectors. These resources include genomes, RefSeq transcripts, placements for genomic, MICEr and gene trap clones, repeats and genomic sequences not included in the assembly. NCBI also provides annotation for several partial assemblies of clone-based sequences from other mouse strains. These annotations can be viewed in the NCBI MapViewer, which permits the simultaneous display of genomic maps with different coordinate systems. We will present the current state of the NCBI clone database and discuss how the database will be updated with new data.

25D

Coordinated efforts for mouse phenotyping within Asia - Establishment of AMPC (Asian Mouse Phenotyping Consortium). Je Kyung Seong1, Ki-Hoan Nam2, Xiaohui Wu4, Jeffrey Yen5, Leo Wang6, Si-Tse Jiang6, Atsushi Yoshiki7, Hisoshi Masuya8, Shigeharu Wakana8. 1) Seoul National University, Seoul, Korea; 2) Biomedical Mouse Research Center, China; 3) Model Animal Research Center, Nanjing University, China; 4) Institute of Developmental Biology and Molecular Medicine, Fudan University, China; 5) Taiwan Mouse Clinic, Academia Sinica, Taiwan; 6) National Laboratory Animal Center, Taiwan; 7) Experimental Animal Division, RIKEN BioResource Center, Japan; 8) Japan Mouse Clinic, RIKEN BioResource Center, Japan.

The mouse has emerged as one of the best models for biological research and human diseases to enable the integrated genetic approach. Tremendous genetic mouse resources, including inbred strains, spontaneous and induced mutants, transgenic lines, and a large resource of knockout lines, are now available for broad community of biomedical research. Nevertheless, capitalizing of these resources crucially depends on the systematic phenotyping through both of the broad-based and in-depth characterization. Standardized protocol and genetic background control are crucial for comparing the data from different laboratories and research centers. Coordinated phenotyping efforts will also avoid the overlapping or duplicated characterization as well as fragmented or inconsistent information. In addition, many of these in-depth analyses require expensive equipments and skilled technical support to form a phenotyping network and ensure the quality of data. Currently, several mouse phenotyping initiatives are underway by acknowledged the value of functional analyzing these mouse models. Several parts of this project plans have been presented in the ASNA, including Taiwan, China, Korea, and Japan.

With the agreement of the cooperative action on the mouse phenotyping, the Asian Mouse Phenotyping Consortium (AMPC) has been established to promote the coordination and development of advanced strategies and service platforms for phenotyping and informatics.
GENOME-WIDE COPY NUMBER VARIATION IN 162 INBRED MOUSE STRAINS.
Jin Szatkiewicz1, Gary Churchill2, Fernando Pardo-Manuel de Villena1, Patrick Sullivan1. 1) University of North Carolina, Chapel Hill, NC, USA; 2) The Jackson Laboratory, Bar Harbor, ME, USA.

Objective: Copy number variants (CNV) are an important source of genetic diversity. We conducted a genome-wide survey of CNVs in 162 strains of inbred laboratory mice using the Affymetrix Mouse Diversity Array (MDA). The mouse strains were selected to canvass four of the major subspecies of Mus musculus (M. m. domesticus, m. musculus, castaneus, and molossinus). Methods: We analyzed hybridization intensity data from 622,955 SNPs and 597,225 exon probe sets and used an integrated Hidden Markov model as implemented in PennCNV to generate an initial set of CNV's calls. We then applied a multi-step quality control procedure to derive the most confident callset and applied three complimentary methods to validate the CNVs. The analytic callset was annotated using multiple genomic and biological databases. We tested co-localization between CNVs and each genomic feature and tested for enriched functional annotation gene categories. Results: We identified 2,309 copy number variant regions (CNVRs). Among them, 15% has CNV minor allele frequency < 0.01 giving median length of 20Kb and spanning 1.5% (39 Mb) of the genome. We identified multiple genes deleted in an inbred strain whose knockout is reported to be embryonic lethal. Conclusion: We have conducted the most comprehensive survey of CNVs in the laboratory mouse. Our CNV catalog provides insights into the genetics of complex traits as well as genome evolution and speciation.

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MOUSE NCBI M37 ANNOTATION IN ENSMBL.
Amy Tang1, Daniel Barrell1, Susan Fairley1, Thibaut Hourlier1, Magali Ruffier1, Michael Schuster1, Simon White1, Amonida Zadissa1, Bronwen Aken1, Jan-Hinrich Vogel1, Steve Searle1, Tim Hubbard1. 1) Wellcome Trust Sanger Institute, Cambridge, United Kingdom; 2) European Bioinformatics Institute, Cambridge, United Kingdom; 3) Dept of Bioinformatics & Computational Biology, Genentech, Inc., San Francisco, CA.

PURPOSE: Ensembl (www.ensembl.org) provides integrated genome annotation for Mouse and other vertebrate species, including coding and non-coding gene annotation, multiple species alignments, gene tree, functional genomics, etc. The Ensembl mouse gene set (Ensembl release 61, Feb 2011) contains 36,814 genes, of which 22,654 are protein-coding. Currently 57% of these protein-coding genes are "merged" (containing Ensembl-Vega merged transcripts). The remaining non-merged gene/protein genes are mainly contributed by Ensembl's genome-wide annotation in regions where HAVANA have not yet provided manual annotation. The Ensembl gene set includes annotation for ~99% of NCBI RefSeq (version 43.0) and UniProt/SwissProt (2010-10 release) curated mouse proteins. In particular, 94.59% NCBI RefSeq proteins were exact matches for translations of Ensembl mouse protein-coding genes. Ensembl collaborates with HAVANA, NCBI and UCSC in the Consensus Coding Sequence (CCDS) project (www.ncbi.nlm.nih.gov/projects/CCDS) which aims at identifying a core set of protein coding regions that are consistently annotated by all collaborating groups and of high quality. To this end, the Ensembl mouse gene annotation from release 61 was used to update the CCDS set, thereby increasing the number of CCDS models from 17,637 coding sequences in 16,684 genes (22 Sept 2010) to 22,158 coding sequences in 19,555 genes (9 Feb 2011).

259B
IMPROVING GENE CLUSTER ANNOTATION ON THE MOUSE REFERENCE ASSEMBLY.
Mark D. Thomas, Clara Amid, Jonathan M. Mudge, Gary I. Saunders, Laurens G. Wilming, Jennifer L. Harrow. Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

In recent years there has been a deluge of mouse genome data, with the release of the finished reference genome in 2009 and more recently the genomes of a further 17 mouse strains by the Mouse Genomes Project. Despite the increasing amount of genomic data available, many regions remain poorly assembled with incomplete gene annotation. These regions often contain gene family clusters that display considerable variation, emphasizing the need for detailed gene annotation based on high quality genome assemblies. The HAVANA group provides high quality manual gene annotation that underlies the human ENCODE and mouse EUCOMM projects. Following established guidelines, we annotate all coding and non-coding loci to produce a comprehensive gene set entirely supported by EST, mRNA and protein evidence. The accurate annotation of splice variants and pseudogenes within gene clusters is particularly important, as these are often misrepresented by automatic annotation methods.

The significance of detailed manual annotation is illustrated by gene clusters such as the MUPs and the defensins. The mouse MUPs cluster on chromosome 259B and worldwide courses keeps us up to date with the data and tools Ensembl users require.

260C
ENSEMBL GENOME BROWSER.
Jana Vondrousova1, Ensembl Team1,2. 1) European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SD Cambridge, UK; 2) Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, Cambridge, UK.

The Ensembl project (http://www.ensembl.org) provides an infrastructure that integrates high quality annotation on chordate and selected eukaryotic genomes. The evidence-based annotation produces unique datasets that contain information for core genomic features such as the Ensembl’s gene set as well as numerous additional data including multiple species alignments, homology prediction, sequence-specific variations, and regulatory annotation. The Ensembl genome browser integrates data from numerous ancillary databases: manually curated gene models from Vega, polymorphisms and other sequence variation from dbSNP, protein annotation from Pfam, InterPro and UniProt, etc. Moreover, Ensembl supports the visualisation of mouse-specific resources including the position of clones from BAC libraries, ditag libraries, knockout and JKMC products. Information about MGI phenotypes, in-situ gene Expression (IMAGE) or EMMA can be added to gene specific pages. Comparative genomics views are based on data from nearly 60 other genomes. They focus on whole genome alignments and predictions of syntenic regions, along with the identification of gene orthology and protein families. Multiple alignments of sequences specific to individual strains are also available allowing a quick determination of genetic differences. Furthermore, Ensembl uses data from the ENCODE project as well as other datasets to predict the location of regulatory features. Ensembl data are accessible through an integrated web site (www.ensembl.org), direct database querying through MySQL, and a set of Perl APIs. Data can also be extracted using BioMart, a powerful generic data-mining tool that allows users to answer complex queries without any programmatic knowledge. Ensembl data are provided freely and can be downloaded as flat files from the ftp site. Feedback from user surveys, our helpdesk, and worldwide courses keeps us up to date with the data and tools Ensembl users require.
261A


We have designed a 7851 SNP marker genotyping array built on the Illumina Infinium platform. Its markers are distributed throughout the mouse genome with an average spacing of 320 Kb and a standard deviation of 178 Kb. The markers were chosen to be maximally informative and maximally independent. This was achieved by selecting SNPs with high minor-allele frequencies (maximizing entropy) and low local pairwise linkage disequilibrium (minimizing mutual information). This design criteria makes the platform optimal for detecting heterozygous regions, while in homozygous regions it allows for optimal discrimination between haplotypes. These optimization criteria are population dependent. In MUGA the allele frequencies and haplotypes were derived from the 8 diverse mouse strains used in the CC—AJ, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HILJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ. MUGA’s low cost and high-sensitivity makes is well suited for use in marker-assisted breeding selection. We have used MUGA to assess the haplotype mosaics of over 750 CC samples, and it is regularly being used to select breeding pairs to accelerate the CC inbreeding process. MUGA has proven to be an extremely useful genotyping platform for DNA from variable molecular weights in a diverse variety of strains including classical and wild-derived inbred strains, mouse cell lines and wild caught mice. MUGA is publicly available from Neogen's Genesek division at http://www.neogen.com/Genesek/.

262B

Sequence based characterization of structural variation in the mouse genome. Binnaz Yalcin1, Kim Wong2, Martin Goodson1, Avigail Agam1, Thomas Keane2, David Adams2, Jonathan Flint1. 1) Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, Oxford, United Kingdom; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom.

We have produced the first complete catalogue of structural variants (SVs) including deletions, insertions, inversions and copy-number gains across the newly sequenced genomes of 17 laboratory and wild-derived inbred strains. We have identified a total of 1.7 M SVs, affecting 1.2% (33Mb) and 3.7% (98.6Mb) of the genome of the laboratory and wild-derived inbred strains, respectively. This has allowed us to address three critical questions: 1) what is the extent and complexity of structural variation (SV) in the mouse genome 2) what are the likely mechanisms of its formation and 3) what are its phenotypic consequences. Our study identified more structural variants than other studies of the mouse genomes and discovered a greater variety of molecular structures than previously reported. We will explain how we went about findings SVs. Our analysis of SV mechanism relied on two important properties: ancestral state of the SV and single-nucleotide level resolution of breakpoint delineation. Sequence features at SV breakpoint revealed some properties that are common with human SV formation, as well as some striking differences. We predicted 41.7% of SV formed through retrotansposition, 31.3% through MMEJ, 13.3% non-MMEJ, 8.4% FosTeS or others, 5.2% SRS, 0.4% SSA and 0.4% NAHR. We also discovered a relationship between SNP at the junction and SV formation. Finally, we found little in common with human SV formation, as well as some striking differences. We predicted 41.7% of SV formed through retrotansposition, 31.3% through MMEJ, 13.3% non-MMEJ, 8.4% FosTeS or others, 5.2% SRS, 0.4% SSA and 0.4% NAHR. We also discovered a relationship between SNP at the junction and SV formation. Finally, we found little in common with human SV formation, as well as some striking differences.

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End-sequencing of C57BL/6N BAC clones. Atsushi Yoshiki1, Takehide Murata1, Takuhito Yamazaki2, Atsushi Toyoda2, Kazuyuki Mekada1, Satoshi Oota2, Yukiko Yamazaki2, Yuichi Obata1, Kunyu Abe1. 1) RIKEN BioResource Center, Tsukuba, Ibaraki 305-0074, Japan; 2) National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan.

Currently, international collaborative projects for knocking out every protein-coding gene are underway, mainly using C57BL/6N embryonic stem (ES) cells. In Japan, several research organizations are generating knockout and transgenic mice using ES cells and fertilized eggs from the C57BL/6N (B6N) substrain, respectively. Recently, many reports have been published regarding the genetic and phenotypic differences between the C57BL/6J (B6J) and B6N substrains, indicating the importance of C57BL/6N strain selection. However, only B6J bacterial artificial chromosome (BAC) clones were publicly available from the BACPAC Resource Center, and B6N BAC clones were not available. In these circumstances, the Gene Engineering Division of the RIKEN BioResource Center (RBRC) generated and publicly distributed B6N BAC clones, the first B6N BAC library in the world. In this study, we conducted end-sequencing of 124,000 B6N BAC clones to enrich their value. High-molecular-weight genomic DNA was extracted from kidney tissue of C57BL/6N mice and introduced into BAC cloning vectors containing an average insert size of 128 kb. Subsequently, we extracted BAC DNA, end-sequenced the BAC clones, aligned the clones on the reference C57BL/6J genome, and registered their end-sequences in the DNA Data Bank of Japan. We published the data via the “Mouse BAC browser” on our website, where scientists can search for and identify BAC clones of interest that are aligned on the reference genome. Our results enable scientists to perform efficient genetic engineering with these B6N BAC clones and ES cells to facilitate studies of gene functions using the pure C57BL/6N genetic background. B6N BAC clones and ES cells are available from the Gene and Cell Engineering Divisions of RBRC, respectively. This study was supported by Genome Information Upgrading Program of the National BioResource Project, MEXT, Japan.
264A Predicting Function and Phenotype: The Mouse Genome Informatics (MGI) database as a resource for computational data mining. Carol J. Bult1, Matt Hibbs1, Yuanfang Guan2, Cheryl Ackert-Bicknell3, Olga Troyanskaya1. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Princeton University, Princeton, NJ.

The Mouse Genome Informatics (MGI) database is the community model organism database for the laboratory mouse. MGI is widely used in the biomedical research community as a knowledgebase of manually curated functional and phenotypic attributes of mouse genome annotations and as a companion of mouse models for human disease. MGI is also a valuable resource for the computational biological and systems genetics community for the development of novel computational approaches for predicting gene function and phenotype. We report here on two related initiatives that use data from MGI in the development of Bayesian integration methods for predicting networks of functionally related genes (http://mouseNET.princeton.edu; Guan et al. 2008. PLoS Comp Bio 4:e1000165) and for predicting phenotypes (http://cbfg.jax.org/phenotype; Guan et al.2010. PLoS Comp Bio 6:e1000991). Our gene function networks predicted several novel interactors for Nanog, an embryonic stem cell pluripotency gene, which were experimentally validated using a proteomic assay. The gene-to-phenotype predictions identified two novel genes related to bone density (Timp2 and Abcg8) that were validated using mouse knockouts. MGI is supported by NIH grants HG000330, HG002273 and HG033745.

265B Haplotype reconstruction from array hybridization intensities. Daniel M. Gatti1, Cheng-Ping Fu2, Karen L. Svenson1, Keith Shepperson1, Fernando Pardo Manuel de Villena3, Leonard McMillan2, Gary A. Churchill2. 1) The Jackson Laboratory, Bar Harbor, ME; 2) University of North Carolina, Chapel Hill, NC.

Genotyping arrays measure the hybridization of genomic DNA fragments to allele specific probes to determine genotypes at single nucleotide polymorphisms (SNPs). Hybridization intensities are converted to genotype calls by a clustering algorithm that assigns each SNP to homozygous (A or B), heterozygous (H), or no-call (N) classes. Downstream analyses rely on the called genotypes. SNP probes that do not conform to the expected pattern of clustering are filtered out and information that is encoded in the hybridization intensity data is lost. We have observed that the distribution of hybridization intensities is reproducible and rich in information that can be used to identify the ancestry of the target sample beyond the information available in genotype calls. We describe an algorithm for reconstructing ancestral haplotypes directly from the intensity data. In addition to extracting more information from individual SNPs, our hidden Markov model based algorithm borrows information from adjacent SNPs to further improve accuracy of haplotype reconstruction. We demonstrate the method by reconstructing the haplotypes on a sample of Diversity Outbred mice that were genotyped using the Mouse Universal Genotyping Array. We estimate the probability of descent from each of the eight founder strains of this population (36 possible genotypes) at 7,854 SNP loci. This method can be applied to other populations and array types, including widely used platforms for genotyping human, dog, cattle and other model organisms.

266C A General Method for Analyzing Diallel Data. Alan Lenarcic1, Gary Churchill2, William Valdar2. 1) Genetics, UNC, Chapel Hill, NC; 2) Jackson Labs, ME.

The diallel is a genetics breeding design for model organisms such as mice that crosses all individuals with each other. When applied to inbred strains, in our examples, the founders of the Collaborative Cross, it characterizes aggregate effects of genetic background on a phenotype, potentially revealing effects of strain dosage, heterosis, parent of origin, epistasis, and sex-specific versions thereof. However, its analysis is traditionally intricate, rendered with exceptions and caveats to a point that has deterred many geneticists from its use. We present a general method for analyzing diallel data that cleanly decomposes the observed patterns of variation into biologically intuitive components, simultaneously models and accommodates outliers, and provides estimates of effects that automatically incorporate uncertainty due to imbalance, missing data and small sample size. Our method is based on a highly efficient Markov chain Monte Carlo exploration of an extensively hierarchical Bayesian mixed model fitted to ordinary experimental data. Application to physiological and psychometric data suggests that preliminary information from the diallel can then drive later crosses by suggesting additional breeding pairs of most statistical value, as well as informing future hypotheses about mechanisms of inheritable effect.

267A Analyzing complex high-dimensional datasets from mouse-phenotyping studies. Tonia Ludwig1,2, Helmut Fuchs1, Valérie Gailus-Durner1, Martin Harbé de Angelis1. 1) GMC, Institute of Experimental Genetics, Helmholtz Zentrum München, Neuherberg/Munich, Germany; 2) Research Group of Biostatistics, Max-Planck-Institute of Psychiatry Munich, Germany.

The generation and phenotypic characterization of mouse models for human diseases is an important step in the understanding of diseases and the development of adequate treatments. However, only a very comprehensive phenotypic analysis of mutant mouse lines enables the detection of phenotypes that are altered as a consequence of a random or a directed mutation.

Therefore, datasets from these extensive studies consist of a very large number of observed variables, while the observed sample size is usually very small. Common approaches for statistically analyzing these high-dimensional data suffer from too restrictive data assumptions as well as from a lack of statistical test power and an increased false positive rate.

Unlike in similar studies from fundamental research, datasets from phenotyping studies moreover consist of different types of variables (e.g. metric, ordinal, nominal, time-independent or -dependent) and are often affected by large standard deviation. In many cases, the variables from these studies are also correlated. These conditions present a severe problem for most of the usual statistical analysis techniques.

In similar situations with high-dimensional datasets, such as genome wide association studies, machine learning techniques (e.g. Random Forest, LASSO, Elastic Net and LogitBoost) have become more and more of interest. We are investigating the applicability of these methods to mouse phenotyping datasets in order to identify variables that are representing an altered phenotype. We are also developing visualization techniques as tools for the detection of altered phenotypes in these datasets.

All investigated approaches provide valuable solutions in solving the above mentioned problems. Our work presents these methods as well as a validation based on examples.

This work was supported by the German National Genome Research Network (NGFN), Grant No. 01GS0850.

268B mRNA sequencing of mouse spermatogenesis uncovers novel meiotic genes and isoforms. Gernady Margolin, Pavel P. Khi1, Marina A. Bellani, R. Daniel Camerini-Otero. Genetics and Biochemistry Branch, NIH/NIDDK, Bethesda, MD.

We sequenced mRNA from testes of juvenile mice to quantify and classify gene expression in spermatogenesis. As part of the analysis, we performed an in silico de-convolution of temporal gene expression profiles yielding cell type-specific gene expression estimates. Comparison of our gene expression with microarray-based studies yields a reasonable agreement - 89% or more of commonly present genes are classified similarly. Moreover, we identify over 1,000 protein-coding genes showing elevated expression during meiosis that have not been either interrogated or classified before in relation to spermatogenesis. This group is significantly enriched in proteins related to microtubule-based movement, response to DNA damage and zinc finger proteins. The sequencing approach allows us to detect the majority of known genes and isoforms and, importantly, find new ones. We detect expression of 91% of known protein coding genes (genes that are not seen in any of our samples are mostly related to receptor activities) and identify about 75% (~153,000) of all known splice junctions. Additionally, we discover 13,000 novel splicing events some of which skip more than 10 exons. Roughly half of the novel alternative splices preserve the reading frame, indicating the existence of potentially important protein forms. We also estimate that there can be thousands of novel alternative polyadenylation sites. Finally, based on exonic expression as well as splicing activity, we find support for 120 computationally predicted gene models.

269C Development of semantic web based database of mammalian bio-resources. Hiroshi Maniwa1, Taeu Takatsuki1, Mikako Saito1, Kazunori Waki1, Nobuhiko Tanaka1, Kazuyuki Mekadi1, Hatsumi Nakata1, Yoko Makita1, Yoko Yoshica1, Shigebaru Wakana1, Tetsuro Toyoda1, Atsushi Yoshik1. 1) RIKEN BioResource Center, Tsukuba, Japan; 2) RIKEN/BS, Yokohama, Japan.

For the effective and accurate dissemination of information, the semantic-web is expected as the next-generation technology to ensure integration using structured metadata such as ontologies. We have worked out the trial to develop semantic-web based database of mammalian bio-resources, mouse mutant strains and cell lines, which are deposited in RIKEN BioResource Center using Scientists’ Networking System (SciNetS) system, a common data incubation system. We are now capturing the information of about 1,200 mouse strains and 2,200 cell lines with biological property information. Currently, we have successfully organized resource records with metadata of public information such as:
Synergistic approach to multiple-QTL genetic dissection of quantitative complex traits in the composite experimental design consisting of an extended RIX cross and F2 generation of their progenitor strains. Alexander V. Osadchuk1, Mikhail S. Diakov2, David C. Airey3, Lu Lu4, David W. Threadgill5, Robert W. Williams6. 1) Institute of Cytology and Genetics, Novosibirsk, Russian Federation; 2) Vanderbilt University, Department of Pharmacology, TN; 3) University of Tennessee Health Science Center; 4) Dept of Genetics, North Carolina State University.

We developed a novel composite experimental design and effective analytical tool for genetic dissection of complex traits regulated by polygenes with epistatic interactions. The design included two datasets: 1) an extended RIX cross consisting of 94 isogenic lines (13 CXB RI strains, 78 RIX diallel F1s generated from CXBs, both progenitor strains, BALB/cByJ, C57BL/6J, and the nonreciprocal CXB F1); and 2) CXB F2 population. The target phenotype was cerebellum weight. The first dataset (CXB RIX) was used to solve dimensionality and linkage. Specifically, multiple regression analysis combined with a beam search procedure was used to determine a set of solutions derived from the optimal segregation model characterized by the minimal number of loci to account for among-line variation relative to within-line environmental noise. Solutions for the optimal segregation model whose model component SDPs were significantly linked to marker loci in the CXB RI set were selected for further characterization. These solutions were used to compare expected and observed histograms in the second dataset (CXB F2) using Monte Carlo method and G test. Solutions that successfully passed this test were used to predict model multilocus genotypes in the CXB F2 dataset and to perform multiple regression analysis of the joint datasets to estimate integral LRS. Finally we developed the composite genome-wide permutation test. In conclusion, we extracted a single multilocus solution that exceeded the 95th percentile permutation threshold. The approach based on two complementary but independent datasets together with the developed analytical tool demonstrates synergistic results and high effectiveness even for a small set of RI strains.

An Integrated Bayesian Hierarchical Model for Multivariate eQTL Mapping (iBMQ). Marie Pier Scott-Boyer1, Arafat Tayeh2, Aurelie Labbe2, Christian F. Descheppe3, Raphael Gottardo4. 1) IRCM and Université de Montréal, Montreal, Canada; 2) Department of Epidemiology, Biostatistics and Occupational Health, McGill University, Montreal, Canada; 3) Fred Hutchinson Cancer Research Center, Seattle, USA.

We developed gene expression levels as quantitative genetic traits, recent studies have combined microarray and genomic data to detect large numbers of expression quantitative traits loci (eQTLs). Originally, eQTL profiling was performed by analyzing each gene one at a time, using the same tools developed for phenotypic QTL mapping. Since the expression levels of all genes are not independent, it would be more appropriate to analyze all genes across all markers simultaneously, but methods should then be designed to account for the high dimensionality of data. Although others have started developing such methods, they differ in the way to deal with the problem of high dimensionality. We therefore developed a model that takes into account a large number G of gene expressions, a large number S of regressors (genetic markers) and a small number n of individuals in what we call a “large G, large S, small n” paradigm. This integrated Bayesian hierarchical Model for multivariate eQTL mapping (iBMQ) incorporates both genotypic and gene expression data into a single model while 1) specifically coping with the high dimensionality of eQTL data (large number of genes), 2) borrowing strength from all gene expression data for the mapping procedures and 3) controlling the number of false positives to a desirable level. To validate our model, we compared the results of simulation studies to those obtained with 3 other previously published methods. ROC curve analyses showed that iBMQ increased the power to detect eQTL “hotspots” while keeping low false positive rates, particularly when the number on n individuals was small. Analysis of a real gene expression dataset obtained from a panel of 24 mouse AXB-IBA recombinant inbred strains also showed that eQTL hotspots detected by iBMQ showed enrichment in genes belonging to particular GO categories.

Detecting novel variants with high density genotyping arrays. Hyuna Yang1, John Didion2, Keith Sheppard2, Fernando Pardo-Manuel de Villena2, Gary Churchill1. 1) Biostatistics and Bioinformatics, Duke University; 2) Carolina Center for Genome Sciences and Lineberger Comprehensive Cancer Center University of North Carolina; 3) Jackson Laboratory.

Hybridization-based genotyping arrays are widely used in genetic mapping analysis for humans and for many model organisms. The Mouse Diversity Array (MDA) is a high-density genotyping array designed to interrogate the genetic loci of the laboratory mouse. MDA is implemented on the same platform as the Affymetrix Genome-Wide Human SNP 6.0 array and is similar to genotyping arrays for dog, horse, cow and other model organisms. We have observed reproducible patterns of hybridization intensity that deviate from expectations for biallelic loci. We demonstrate that these Variable Nucleotide Oligonucleotide probes (VNOs) result from off-target variants that interfere with hybridization. We have developed new software for genotype calling that can identify VNOs, which results in higher call rates and reduced error frequency. The ability to detect and accurately categorize off target variants provides an important source of new genomic information that avoids the biases induced by SNP selection in genotyping arrays. We observed and validated VNOs in mouse and human genotyping data on both Affymetrix and Illumina platforms. We conclude that VNOs represent a general feature of all hybridization genotyping arrays which can provide useful information beyond what is available in called genotypes.

Bayesian Collaborative Cross Toolkit: a computational framework for modeling haplotype effects in multilocus parent lines. Zhaosun Zhang1, Wei Wang1, William Valdar2. 1) Department of Computer Science, UNC Chapel Hill, Chapel Hill, NC; 2) Department of Genetics, UNC Chapel Hill, Chapel Hill, NC.

The Collaborative Cross (CC) is a new mammalian resource for system genetics, including nearly 200 recently established mouse lines derived from 8 known inbred strains. Here we describe a Bayesian Collaborative Cross Toolkit for modeling the effects of founder haplotypes in CC and other multiparent line data. Our aim to provide a framework for coherent estimation of strain effects that incorporates uncertainty in haplotype assignment, uncertainty in other parameters, models possible effects of dominance (for non-inbred subjects), and that provides a means to incorporate data that may be incoherent structural. Building on the Bayesian modeling toolkit, JAGS, we provide adapted modules and packages that enable us to build a sophisticated model of QTL action. Incorporating founder information from both genotype and phenotype data, we use Gibbs sampling to sample from posterior distributions of unknown variables in the model efficiently. We will present a model estimating haplotype effects in CC data and novel ways to visualize posterior distributions of underlying founder strain effects. We will also show how in our model we take the results of a probabilistic haplotype reconstruction as prior information in order to obtain posterior distributions of associated and predicted QTL effects. We will present our results on simulated CC data, and real qualitative (white spot) and quantitative (immune cell count) phenotypes. Our results demonstrate that the method works well on CC data, even when applied to a small set of lines, and our visualization results on posterior distributions of strain effects clearly show underlying effects patterns and help users to better understand how genetic factors affect phenotypes.

A genome-wide molecular network analysis was enabled by functional annotations using biological ontologies in the mouse cDNA encyclopedia project and other innovations. Since discoveries of new drug targets are expected in disease research using the molecular network information, applications of those information are regarded. Meanwhile, mutant mouse resources have been expanded by the coordinated production of knockout mouse for all coding genes promoted by the International Knockout Mouse Consortium and other many facilities. For this reason, we developed the system designed to automatically annotation and useful search for those resources using ontology and classified terms related to molecular network information of disease, drug target discovery, phenome and other life science researches. The outline of methods about the annotation of mouse resources using disease and drug target information in Kyoto Encyclopedia of Genes and Genomes (KEGG) is as follows. (1)Extraction of mouse strains and allele symbol information from International Mouse Strain Resource database (IMSR). And, mapping gene symbols to the strains using allele symbol, BRC animal search site and other information. (2)Extraction of disease or drug information-associated human or orthologous mouse gene/pathway information from KEGG and MGI site. (3)Converting of synonyms to representative gene symbols. (4)Mapping of experimental mouse strains to disease and drug information using gene symbols. In this study, out of 3030 mouse strains in RIKEN BioResource Center on IMSR data, 531 strains were mapped to disease information and 545 strains were mapped to drug target information via pathway. In contrast, 294 strains were mapped to disease information and 94 strains were mapped to drug target information via disease gene. Our system utilized not only single gene but also the pathway is more effective for survey of candidate experimental mice related to disease, drug target and other ontologies.
POSTER: Gene-environment interactions

Abstract number is above title. The first author is the presenter. Schedule Available at mousegenetics2011.org

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THE EFFECT OF EARLY HOUSING ENVIRONMENT ON ANXIETY, DEPRESSION AND SOCIAL MOTIVATION. Lisa M. Tarantino1, Sheryl S. Moy1, Darla R. Miller2, Patrick F. Sullivan1, Fernando Pardo-Manuel de Villena2. 1) Department of Psychiatry, University of North Carolina, Chapel Hill, NC; 2) Department of Genetics, University of North Carolina, Chapel Hill, NC.

Complex neuropsychiatric syndromes like anxiety, depression and schizophrenia have a significant genetic component but can also be substantially influenced by environmental factors. The Collaborative Cross (CC) population of mice offers the ability to study the role of gene by environment interactions and their effect on rodent models of psychiatric disease in a genetic reference population that more closely approximates the genetic heterogeneity observed in the human population. We have initiated a project to study the role of early housing environment in CC founder strains on behavioral models of anxiety, depression, schizophrenia and asocial behavior. Mice from five founder strains and reciprocal F1s were exposed to standard group housing, isolate housing or enriched housing for five weeks from weaning. At eight weeks of age, the mice were tested in a number of standard rodent behavioral assays. The data from these studies indicate that enriched housing results in decreased anxiety and increased locomotor response to novelty in the open field and isolate housing decreases social preference in female, but not male mice. Significant strain differences were also observed for most behaviors. These data demonstrate a role for both early environment and genetics on psychiatric disease related behaviors in the CC founder strains and provide a platform on which to expand our studies into the more informative CC population.

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EPIGENETIC EFFECTS ON LOCOMOTOR ACTIVITY IN RECIPROCAL F1 MICE. Amy F. Eisener-Dorman1, Joseph Farrington1, Fernando Pardo-Manuel de Villena2, Lisa M. Tarantino2. 1) Department of Pathology, University of Washington, Seattle, WA; 2) Department of Medicine, University of Washington, Seattle, WA.

Epigenetic modifications that modulate homeobox (Hox) gene expression are important in regulating developmental processes. Histone methylation has been implicated in the temporal control of expression of genes within the Hox cluster, thereby providing an important role in establishing body patterning. Genes in the Hox (reproductive homeobox on the X chromosome) cluster on the mouse X chromosome are expressed in early development and in a sexually dimorphic manner with Rhox6 and 9 expressed higher in ovary compared to testis. We report that KDM6A, which removes trimethylation at lysine 27 of histone H3 (H3K27me3), regulates Rhox6 and 9 expression. KDM6A specifically binds to both genes during early female ES cell differentiation, resulting in removal of H3K27me3 and increased expression, a process inhibited by KDM6A knockdown. In contrast, Rhox expression does not increase during differentiation of male ES cells and KDM6A knockdown does not affect Rhox expression in these cells. Differentiation into cell types where Rhox6 and 9 are no longer expressed is associated with loss of KDM6A binding. Moreover, in ovary where the genes are highly expressed, KDM6A binds to both Rhox genes. Our study implicates for the first time a gene that escapes X inactivation, Kdm6a, in the regulation of homeobox genes involved in reproduction during early development and in a female-specific tissue.

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The histone demethylase KDM6A regulates Rhox gene expression in early mouse development and in ovary. Joel Berletch1, Xinxin Deng1, Christine Distech1,2. 1) Department of Pathology, University of Washington, Seattle, WA; 2) Department of Medicine, University of Washington, Seattle, WA.

Histone demethylases (KDM6) are a class of proteins that remove trimethyl groups from histone lysine 27 (H3K27me3). We previously reported that KDM6A removes trimethylation on H3K27me3 and that KDM6A binds to both Rhox6 and Rhox9 on the female X chromosome. We also found that Kdm6a expression is altered in females of a genetic knockout model of KDM6A. The present study aims to determine where and when KDM6A regulates the expression of Rhox genes, and whether KDM6A affects Rhox expression in females in a cell-specific manner. Since KDM6A is a histone demethylase, we hypothesized that KDM6A regulates Rhox expression by removing methyl groups from histone H3. To test this hypothesis, we performed ChIP-Seq analysis on the males to identify genes that are differentially methylated. We believe the results of these studies will have significant implications for the genetic mechanisms and neurological pathways underlying locomotor response to novelty.
The Y chromosome influences experimental allergic encephalomyelitis susceptibility in male and female C57BL/6J Y chromosome consomic mice. Laure Case1, Emma Wall1, Naresha Saligrama1, Elizabeth Blankenhorn2, Cory Teuscher1. 1) Medicine, University of Vermont, Burlington, VT; 2) Microbiology and Immunology, Drexel University, Philadelphia, PA.

Experimental allergic encephalomyelitis (EAE) is the autoimmune animal model for multiple sclerosis and provides researchers with a system that closely parallels the human disease. We have identified a Y Chr-mediated effect, termed Yeae, that influences EAE susceptibility in both male and female progeny using C57BL/6J Y-Chr consomic mice (B6.Y). One potential source for this Y Chr-mediated effect on female progeny is exposure to their brothers in utero, who may transmit male-specific factors during gestation. In the male fetus, Sry (sex determining region Y) initiates the bipotential gonad to differentiate into Sertoli and Leydig cells and fetal Leydig cells produce the androgens required for masculinization of the male during embryogenesis. Therefore, we hypothesized that polymorphisms in Sry may regulate EAE susceptibility in males and the organizational masculinization of EAE in females due to differences in the timing and/or intensity of prenatal testosterone leading to females exhibiting equal susceptibility to EAE as males. To test our hypothesis, we used a panel of 15 B6.Y consomic mice in which functionally significant Sry polymorphisms are well documented, to compare the strain distribution pattern (SDP) of Sry polymorphisms with the SDP of EAE susceptibility in male and female mice. While certain strains showed decreased susceptibility in both males and females, our analysis of the consomic lines revealed that the SDP of EAE susceptibility is discordant with the SDP of Sry alleles. These data suggest that Sry and allele dependent variations in the prenatal testosterone surge may not be responsible for reduced EAE susceptibility. Future studies will focus on the only other possible contributors of the Yeae-mediated effect on female mice, including exposure to their father and brothers during the neonatal period or through semen, and on identifying the Y-Chr determinants responsible for this phenotype.

Transgenerational Epigenetic Programming by a Maternally-deposited Mouse Polycomb Group Protein. Sundeep Kalantry, Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Cells retain their identity in part by inheriting gene expression profiles of their predecessors. Patterns of gene expression transmitted through cell division often propagate through modifications of histones and DNA, and do not involve changes in the DNA sequence. Emerging evidence implicates this mode of inheritance, referred to as epigenetic, in a myriad of developmental processes and in human disease. Alterations in the epigenetic machinery are a powerful means to change the expression of a wide variety of genes in a long-term manner. Although much is known about the molecular mechanisms that stably transmit gene expression states through mitosis, the factors that mediate epigenetic inheritance through meiosis in mammals is only scantily known. Meiotic, or transgenerational, epigenetic inheritance is being recognized to play a novel role in the transcriptional programming during mammalian embryogenesis whose effects can persist into adulthood. Here we test the function of oocyte-derived methyltransferase protein, EZH2, as a transgenerational factor required for the establishment of epigenetic gene expression patterns leading to the proper development of the early mouse embryo. EZH2 is a member of the Polycomb group and is thought to propagate transcriptional silencing by locus-specific methylation of histones. We and others have previously shown that the Polycomb group complex whose activity is catalyzed by EZH2 epigenetically maintains mouse X-chromosome inactivation and embryonic stem cell self-renewal. To determine if the Polycomb group not only maintains but also triggers epigenetic transcriptional states, we tested if oocyte-derived EZH2 protein initiates epigenetic transcriptional silencing in the early mouse embryo. Our data indicate that maternally-deposited EZH2 is: 1) dispensable for initiating mouse imprinted X-inactivation; and 2) required for the proper specification of early embryonic lineages in a dose-dependent manner. These data suggest a finely-tuned role for the Polycomb group in transgenerational epigenetic programming of the early mouse embryo.

Structural destabilization of Y chromosomes in interspecific backcrosses is consistent with variations in epigenetic modifications. Ferez S. Nallaseth1,2, Z. Sheng Guo1,2, 1) Dept Mol Biol & Bioch, Rutgers Univ/Ctr Advan Biotech and Med, Piscataway, NJ 08854; 2) Huffington Ctr on Aging and Dept of Mol and Cell Biology, Baylor College of Medicine, Houston, TX 77030; 3) University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA.

The Y chromosome (Ysw) from Mus domesticus poschiavinae when backcrossed into the C57BL/6J inbred strain genome undergoes structural destabilization and functional inactivation; it is stable in the 129/Sv genome (Eicher et al, 1982; Nallaseth, 1992). Branched molecules migrate slower than linear double stranded molecules of equivalent molecular weight under agarose gel electrophoretic conditions (Bell & Byers, 1982). Characteristic electrophoretic mobility shifts of genomic copies of Y-linked repeated sequence restriction endonuclease fragments resulted from the formation of branched molecules since the native species was resistant to concentrations of Mung Bean Nuclease which digested the mobility shifted species to extinction. Variable numbers of genomic copies of Ysw-linked repeated sequences of High Copy (HC) or Xsw(Ysw) female littersmates migrated as the mobility shifted species. Sandwich Southern blots involving transfer through nitrocellulose to nylon membranes, identified Y-chromosomal sequences that were tightly/covalently bound to peptides; similarly to those previously identified in the genome (Neuer et al, 1983; Tse et al, 1980; Keeney et al, 1997). The pl and location of these peptides can be expected to affect the stability of the DNA duplex. Denaturation of restricted genomic DNAs from IC siblings under conditions of constant temperature of 56°C but incremental pH, identified Ysw fractions that displayed distinct melting profiles and mobility shifts. These results are consistent with variations in epigenetic modifications, including methylation patterns and qualitative and quantitative linkage of peptides to sequence elements on the Ysw chromosome. Strain dependent variations in modifications could affect duplex stability and XY heteroduplex formation, which on resolution, lead to uncoupling of Ysw chromosome linkage.

Genome-wide identification of maternal imprinting control regions by MeDIP-seq. Reiner Schulz1, Charlotte Proudhon1, Rachel Duffié2, Guillermo Carbajosa3, Michelle Holland3, Wardhman Rakyam1, Deborah Bourc’his2. 1) King’s College London, Medical and Molecular Genetics, UK; 2) Institut Curie, UMR3215/Inserm U934, Paris, France; 3) Queen Mary University, Blizzard Institute, London, UK.

Genomic imprinting is the differential epigenetic marking of the parental genomes. Primarily, these differences are established in the parental germ lines where specific regions of the genome, imprinting control regions (ICRs), undergo heritable DNA methylation during either oogenesis or spermatogenesis. In the offspring, ICRs cause genes under their control to be parent-of-origin-specifically expressed, which is vital for normal development. In the mouse, 20 ICRs have been identified, controlling ≈80 genes. All but 3 ICRs are methylated on the maternal allele. These maternal ICRs are all associated with CpG island promoters. We aimed to identify all maternal ICRs in mouse. Methylation of maternal ICRs in the oocyte depends on Dnmt3l. We compared the DNA methylation profiles generated by MeDIP-seq of mutant offspring of Dnmt3l1−/− females and wildtype males with the profiles of their wildtype litter mates at 8.5dpc. This identified 76 regions that were differentially methylated (FDR<10%); 52 were hypo-methylated in the mutants, including all but 2 of the known maternal ICRs. The paternal ICRs H19/IGF2, Dlk1/Gtl2 and Rasgrf1 were unaffected. The paternally methylated region at Gpr1/2zhd2 was hyper-methylated. Few of the candidate regions were associated with promoters, CpG islands, imprinting-typical histone modifications or hypo-methylation in sperm. So far, Dnmt3l-dependent methylation in oocytes and maintenance of maternal-only methylation during pre-implantation were confirmed for 4 candidates. One fulfills all criteria of a maternal ICR, but 3 regain paternal methylation: two at implantation and one tissue-specifically in adults. We conclude that most maternal ICRs that are maintained in the adult have been identified. The functional significance of maternal ICRs that persist only during early development remains to be determined.

A genome-wide molecular network analysis was enabled by functional annotations using biological ontologies in the mouse cDNA encyclopedia project and other innovations. Since discoveries of new drug targets are expected in disease research using the molecular network information, applications of those information are regarded. Meanwhile, mutant mouse resources have been expanded by the coordinated production of knockout mouse for all coding genes promoted by the International Knockout Mouse Consortium and other many facilities. For this reason, we developed the system designed to automatically annotation and useful search for those resources using ontology and classified terms related to molecular network information of disease, drug target discovery, phenome and other life science researches. The outline of methods about the annotation of mouse resources using disease and drug target information in Kyoto Encyclopedia of Genes and Genomes (KEGG) is as follows. (1) Extraction of mouse strains and allele symbol information from International Mouse Strain Resource database (IMSR). And, mapping gene symbols to the strains using allele symbol, BRC animal search site and other information. (2) Extraction of disease or drug information-associated human or orthologous mouse gene/pathway information from KEGG and MGI site. (3) Converting of synonyms to representative gene symbols. (4) Mapping of experimental mouse strains to disease and drug information using gene symbols. In this study, out of 3030 mouse strains in RIKEN BioResource Center on IMSR data, 531 strains were mapped to disease information and 545 strains were mapped to drug target information via pathway. In contrast, 294 strains were mapped to disease information and 94 strains were mapped to drug target information via disease gene. Our system utilized not only single gene but also the pathway is more effective for survey of candidate experimental mice related to disease, drug target and other ontologies.
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