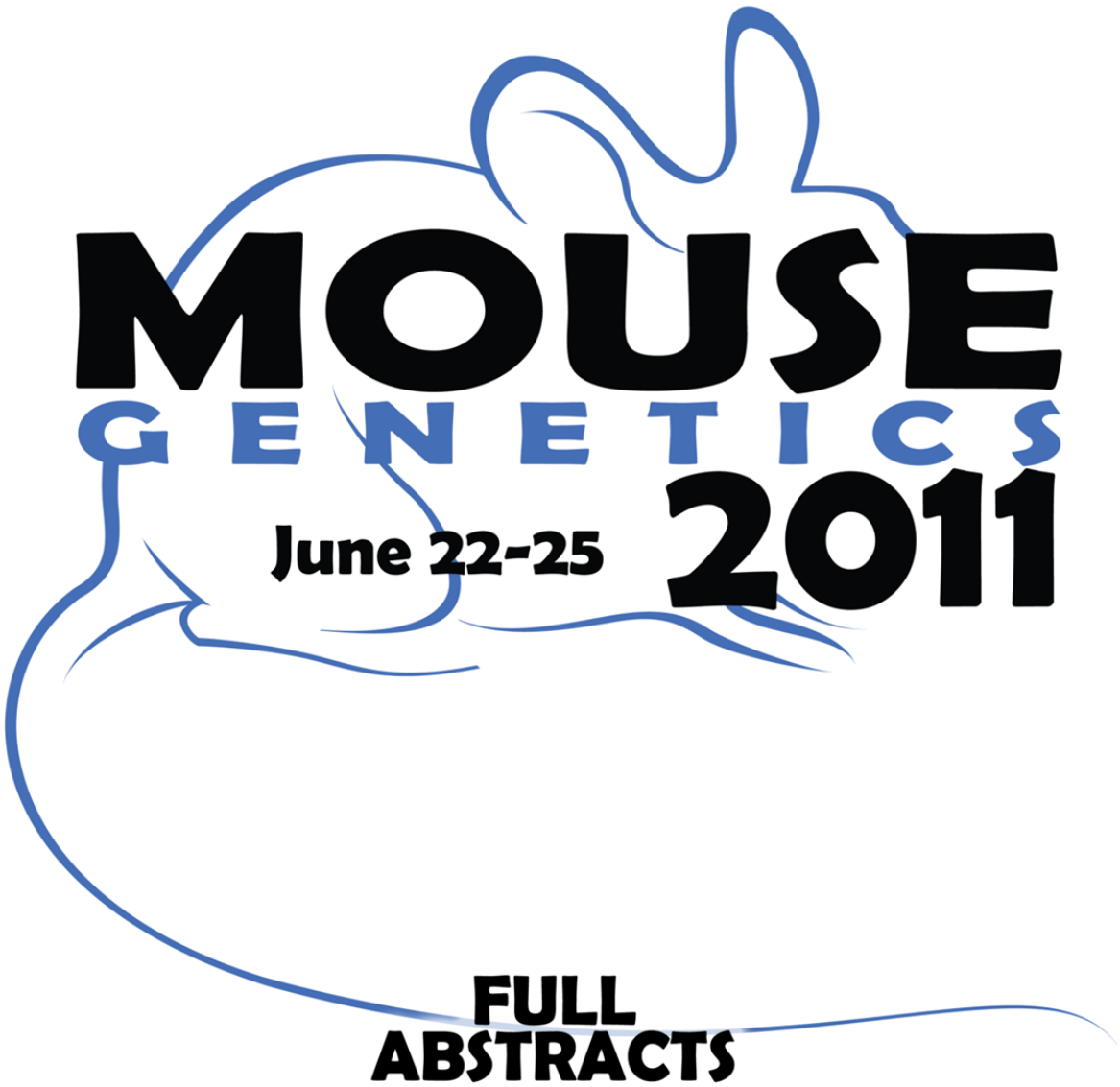
The Genetics Society of America Conferences



FULL ABSTRACTS

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Using Mice to Track Stem Cells in Tissue Morphogenesis and Cancer. Elaine Fuchs. The Rockefeller University, New York.

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Human Genetics After Large-Scale Genome Sequencing. Han Brunner. Radboud University Nijmegen Medical Center, Netherlands.

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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 elucidate 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.

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TBA. David Valle. Johns Hopkins University, Baltimore, Maryland.

Student Symposium

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

1

A cell-based screen for genes involved in mammalian cilia formation and function. Emma A. Hall, Pleasantine Mill, Ian J. Jackson. MRC Human Genetics Unit, Edinburgh, Scotland, United Kingdom.

Primary cilia are essential for mouse development, and are important for key signalling events, particularly Hedgehog (Hh) signalling. Mouse mutants 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 cross-species analysis plus proteomic and transciptomic 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. Eva[1,4], Kyoko E. Yuki[1,4], Shauna M. Dauphinee[1,4], Etienne Richer[5], Silvia Vidal[1,3,4], Danielle Malo[1,2,4]. 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) BioMedCom 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 chemical ENU mutagenesis screen to identify novel genes affecting the host response to *Salmonella in vivo*. The recessive screen for *Salmonella* susceptibility in G3 ENU-mutants was done using mutagenized 129S1 mice (G0). 129S1 mice are resistant to *Salmonella* Typhimurium infection and do not carry any major *Salmonella*-susceptibility genes. 129X1 or DBA/2J mice were used in the breeding of G3 mice to introduce genetic diversity to allow the mapping of the mutant phenotype directly in G3 mice. We have screened 5,638 G3 mice corresponding to 319 G1 mice and identified 7 mutant pedigrees. We have confirmed and mapped 4 of these mutants: *Ity9* (Chr 6), *Ity14* (Chr 1), *Ity15* (Chr 15) and *Ity16* (Chr 8). Two of these loci have been identified and exert their effect through two different mechanisms: *Ity9* mice carry a single point mutation (L361F) in the gene encoding USP18 (ubiquitin specific peptidase 18) affecting Type 1 interferon signaling and *Ity16* mice carry a nonsense mutation within the gene *Ank1* (ankyrin 1), affecting red blood cell turnover and iron metabolism. ENU mutants are further characterized for various phenotypes known to be related to *Salmonella* susceptibility including bacterial load in tissues, clinical hematology, pathology, cytokine/chemokine profile in serum, as well as immunophenotyping by flow cytometry during infection. A better understanding of cellular types and pathways influenced by Ity^{ENU} loci during *Salmonella* infection will help their identification and our understanding of their role in the pathogenesis of the disease.

3

Specific fatty acids exert differential effects on inflammation and intestinal neoplasia. Stephanie K. Doerner[1], Elaine S. Leung[3], Nathan A. Berger[2], Joseph H. Nadeau[3]. 1) Genetics, Case Western Reserve University, Cleveland, OH; 2) Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; 3) Institute for Systems Biology, Seattle, WA.

Obesity and a high fat diet (HFD) increase risk of colon cancer, but their close epidemiological association complicates testing their independent contributions to intestinal carcinogenesis in humans. To test whether a HFD promotes polyp development, independent of obesity, we used Chromosome Substitution Strains (CSSs) in combination with the B6.$Apc^{Min/+}$ mouse model of intestinal neoplasia to generate Apc-CSSs that were either susceptible or resistant to diet-induced obesity. We tested three calorically balanced diets that contained 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 obesity, (3) If fatty acids in the diet are key, then high polyp numbers will be found with some but not all HFDs. Polyp numbers were significantly increased in all strains fed the HFD_{Coco} and HFD_{Corn} for 60 days, regardless of obese or lean status. Interestingly, this increase was not observed after similar exposure to the HFD_{Olive}. Short-term exposure to these HFDs was sufficient to significantly increase polyp numbers and these polyp numbers also stabilized after 30 days. Circulating levels of adiponectin were decreased, while leptin, IL-1B and IL-6 were increased in B6.$Apc^{Min/+}$ fed the HFD_{Coco} or HFD_{Corn} compared to mice fed the corresponding LFDs. With the novel Apc-CSSs, we demonstrate that a short exposure to high levels of specific dietary fats is sufficient to induce both an inflammatory response and an increase in polyp number, and that these effects are most likely 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.

4

Fine-Mapping QTL and Inferring Causal Pathways that Underlie Sixty Murine Phenotypes. Jon Krohn[1], Felix Agakov[2], Paul McKeigue[2], Jonathan Flint[1]. 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 EH8 9AG, 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 almost invariably contain several genes and regulatory regions. Eric Schadt and his colleagues have published many high-impact papers (e.g., Schadt et al. 2005 Nat Genet 37: 710-7) describing their Likelihood-Based Causality Model Selection, which they claim enables the identification of QTGs via the inference of three-level causal pathways from genetic variation (e.g., single nucleotide polymorphisms, or SNPs) to gene expression (i.e., mRNA) levels to a phenotype. However, Schadt and coworkers' methodology does not take into account the critical confounding influences of additional factors -- both biological and environmental -- upon their three-variable causal models. We have developed a Bayesian machine learning technique, called Sparse Instrumental Variables (SPIV), that builds extensively upon Schadt and colleagues' work by allowing for the impact of observed and unobserved variables upon causal pathways, and we show that their causal pathway findings do not hold up in those more rigorous circumstances. Subsequently, we apply SPIV to whole-genome SNP and gene expression data collected from 400 heterogeneous stock mice in order to fine-map QTL and infer causal pathways that underlie 60 diverse phenotypes, including anatomical, biochemical and immunological traits, as well as mouse models of anxiety, asthma and diabetes.

5

Mapping and identification of suppressors of MECP2 symptoms in the mouse. Christie M. Buchovecky[1], Frank J. Probst[1], Hannah M. Brown[1], Jill K. Crowe[1], Jay A. Shendure[2], Monica J. Justice[1]. 1) Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Department of Genome Sciences, School of Medicine, University of Washington, Seattle, WA.

Rett Syndrome (RTT) is a severe neurological disease that presents with developmental regression including concomitant autistic features. Mutations in the methyl CpG binding protein 2 (*MECP2*) cause more than eighty percent of cases. *Mecp2* function has been eliminated in a RTT mouse model, recapitulating many aspects of the human disease. Studies of this mouse model have shown that symptoms can be reversed by introduction of *Mecp2* and partially rescued with other factors, providing substantial evidence that therapeutic intervention is possible. Unfortunately, as a potent transcription factor, MECP2 levels are extremely dosage sensitive, making direct manipulation of it a poor treatment option. Therefore, we have employed a random mutagenesis, dominant screen in the mouse model to identify secondary molecules and pathways that are important for suppression or amelioration of RTT symptoms. So far, we have mapped five modifier loci to different chromosomal locations. Use of sequence capture technologies followed by NextGen sequencing has allowed us to identify candidate genes in three of these loci. My project focuses on identifying the basis for suppression in one mouse line that shows near complete rescue of the RTT phenotype, but segregates two independent modifiers. Understanding these modifiers at the molecular level will highlight potential drug treatments that may reverse RTT symptoms in children suffering from the disease.

6

Vacuolated lens (*vl*): a multigenic mouse mutant model of Neural Tube Defects (NTDs). Bo Li[1,2], Paul G. Matteson[1], Alejandro Q. Nato, Jr.[3], Tara C. Matise[3], James H. Millonig[1,2,3]. 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 vacuolated 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 folds. Crossing vl^{C3H} mice to the MOLF/EiJ strain rescues the *vl* defects. QTL analysis mapped three modifiers (*Modvl*: **Mod**ifier of ***vl***). One QTL being studied is *Modvl5* (LOD=5.0; Chr18). $Modvl5^{MOLF}$ congenic was generated to study whether this locus is sufficient to rescue the *vl* phenotypes. Congenic embryos at different development stages were genotyped and *vl*-associated lethality was determined to occur between E8.5 and E9.5. $Modvl5^{C/M}$ is sufficient to rescue the lethality

(p=0.004). Morphological analysis indicates *Modvl5*$^{C/M}$ partially rescues the *vl*-associated NTDs (p=0.03). Bioinformatics analysis determined the transcription factor, *Cdx1*, is the only gene within the *Modvl5* 95% CI co-expressed with *Gpr161* in the neural folds. Re-sequencing Cdx1 identified a poly-glutamine polymorphism (5Q-C3H, 7Q-MOLF) predicted to affect secondary and tertiary protein structure. Additional analysis determined BALB/cJ has the 5Q allele and does not rescue the *vl* defects, while PWK/PhJ has the 7Q allele and rescues the phenotypes (p=0.0001). Finally, a *Cdx1* binding site is situated at -13 in the *Gpr161* promoter, suggesting *Cdx1* regulates *Gpr161* expression during development and may be responsible for the phenotypic rescue. These results establish *vl* as one of the first multigenic mouse models of NTDs and demonstrate that *Cdx1* is a modifier for *Modvl5*.

7

The role of meiotic sex chromosome inactivation in sterility of interspecific hybrids. Tanmoy Bhattacharyya[1], Ondra Mihola[1], Petr Šimeček[1], Mary Ann Handel[2], Jiří Forejt[1]. 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, *Hst1/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 m. musculus* and *Mus m. 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 H1t-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 (MSUC), 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.

8

Fuz, a Planar Cell Polarity Gene Coordinates Signaling Pathways During Craniofacial Development. Zichao Zhang[1], Bogdan Wlodarczyk[2], Karen Niederreither[2], Shankar Venugopalan[1], Sergio Florez[1], Heather Szabo-Rogers[3], Karen Liu[3], Richard Finnell[2], Brad Amendt[1]. 1) CEGM, Texas A&M - Health Science Center - IBT, Houston, TX; 2) Dell Pediatric Research Institute, University of Texas, Austin, TX; 3) Department of Craniofacial Development, King's College London, London, UK.

Background: The planar cell polarity effector gene *Fuz*, regulates ciliogenesis and *Fuz* loss of function studies reveal an array of embryonic phenotypes. However, cilia defects can affect many signalling pathways and, in humans, cilia defects underlie several craniofacial anomalies. To address this, we analyzed the craniofacial phenotype and signaling responses of the *Fuz*-/- mice. Results: We demonstrate a unique role for *Fuz* in regulating both HH and WNT signaling during craniofacial development. *Fuz* expression first appears in the dorsal neural tissues and later in ventral tissues and craniofacial regions during embryonic development coincident with cilia development. The *Fuz*-/- mice exhibit severe craniofacial deformities including anophthalmia, agenesis of the tongue and incisors, a hypoplastic mandible, cleft palate and hyperplastic miss-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 expression of WNT pathway genes as well as a WNT-dependent reporter. Finally, chromatin IP experiments demonstrate that β-catenin/TCF-binding directly regulates expression of *Fuz*. Conclusions: These data demonstrate a new model for coordination of HH and WNT signalling and reveal a FUZ-dependent negative feedback loop controlling Wnt/β-catenin signalling.

9

A population-based model for mapping quantitative trait loci for susceptibility to environmentally-induced diseases. Michelle DeSimone[1], Alan Bohn[1], Garrison Glavich[1], Katherine McCaffery[2], Heather Patisaul[2], David Threadgill[1]. 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 intercross population derived from FVB/N-*Mdrb1a/1b*-/-, a multi-drug resistant p-glycoprotein knockout mouse model, and CAST/EiJ a wild-derived strain that is genetically distinct from the FVB/N background. By modulating chemical transport and genetic variation in the mouse to model human biology, including using a western diet, environmentally-relevant doses can be evaluated for their effects on toxicity susceptibility. A study cohort of 900 F3 mice was divided into nine dose groups, each containing 50 female and 50 males, and was administered suspect carcinogens and neurotoxicants, trichloroethylene and sodium arsenite, via the drinking water and chow, respectively, at environmentally-relevant concentrations for 52 weeks using a dose-ratio approach. We collected serum and urine in metabolic cages at week 0, 16 and 42 for evaluation of biomarkers of kidney and other organ injury, and tested mice for neurological defects. Levels of classical injury markers serum alanine aminotransferase and blood urea nitrogen showed no significant differences between dose and treatment groups at 16 weeks. However, urinary neutrophil gelatinase-associated lipocalin-2, a sensitive biomarker of early renal injury, was increased 3- to 7-fold over controls at week 16 in mice treated with both TCE and iAs. This data suggests that environmental carcinogens may cooperate to produce renal toxicity that may be modulated by genetic determinants.

10

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 and in an *in vivo* transgenic 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 anaylsis several binding sites for CTCF, the mammalian insulator protein, surrounding the *Trpm5* promoter. The candidate CTCF binding sites have insulator function in an *in vitro* reporter assay. Chromosome conformation capture will allow us to visualize the potential interactions between these sites to interrogate the hypothesis that *Trpm5* is protected from silencing by *Lit1*.

11

Pleiotropy Involving Skeletal, Vascular, and Reproductive Phenotypes:Convergence via Endothelin Signaling and Nos3 Activity. jasmin kristianto, jacqueline fisher, Michael Johnson, Zhijie Wang, Chen-yen Ooi, Suzanne Litscher, Naomi Chesler PhD, Robert Blank MD PhD. medicine, University of wisconsin- Madison, madison, WI.

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-8 and HcB-23, our laboratory has identified a pleiotropic quantitative trait locus (QTL) on mouse chromosome 4 that modulates skeletal modeling in response to mechanical loading. Recombination during the strains' construction limits the QTL region to ~6.5 Mb and includes ECE1, encoding endothelin converting enzyme 1. We hypothesize that the same QTL mediates modeling differences in response to mechanical loading in both bone and blood vessels and that *Ece1* is the gene underlying the QTL. Further, we hypothesize that deficient vascular modeling in HcB-8 mice leads to impaired reproductive performance. HcB-8 carotid arteries have a smaller diameter at 90 mm Hg and are less compliant to increases in luminal pressure than HcB-23 carotids (p=0.026, p=0.036 respectively). HcB-8 mice

also have larger fractional heart sizes (0.71 ± 0.02% v 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 smaller than HcB-23 litters (4.4 + 1.8 v 5.8 + 2.0, p< 10-3) and HcB-8 pups are lighter at birth (1.2 + 0.2 g v 1.5 + 0.2 g, p <10-20). Placental 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 *Sox10^{Dom}* Model of Hirschsprung disease (HSCR).** Melissa A. Musser[1,2], Jennifer C. Corpening[1], E. Michelle Southard-Smith[1,2]. 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 colons 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 *Sox10$^{Dom/+}$* 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 *Sox10^{Dom}* 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. Elso[1], Sean T. Ivory[1], Michelle Ashton[1], Tony Papenfuss[2], Tom C. Brodnicki[1]. 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, Sjögren's syndrome, and thyroiditis. 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, indicating a potentially mutagenic event. Ten GFP^{+} mice have been identified, confirming that the SB tagged-mutagenesis 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 *Serinc1*, 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-mutagenesis 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 Wang[1], Hyuna Yang[2], Fernando Pardo-Manuel de Villena[1], Gary Churchill[2], Leonard McMillan[1]. 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 reassigned colors to better 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. John Simmons, J. Patel, A. Michalowski, P. Sullivan, S. Zhang, K. Zhang, M. Kuehl, B.-R. Wei, M. Simpson, O. Landgren, 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 functional alterations in the PI3K/MTOR and cyclin/CDK/CDKI/Rb (Rb) pathways are common in MM. Single agent pharmacologic targeting of these pathways, with MTOR inhibitors (rapamycin) or histone deacetylase (HDAC) inhibitors to modulate the Rb pathway, has proven unfruitful in treating MM. To this end, we have investigated whether combining MTOR and HDAC inhibitors might be more effective. Combining the HDAC inhibitor MS-275 with rapamycin had significant synergy *in vitro* and *in vivo*, and accentuated the target effects of both compounds. The combination ameliorated the oncogenic activation of the AKT/MAPK pathway associated with single-agent rapamycin treatment and enhanced apoptosis. To further define the synergistic mechanism, gene expression profiles of the single agents and the combination were analyzed, and revealed the contribution of the individual drugs to the expression pattern observed in the combination. Expression levels of 97 genes after combination treatment appeared to be the result of additive/interactive effects of the individual agents. Pathway analysis of the 84 significant "additive" genes showed expected enrichment in cell cycle pathways, but also revealed unexpected overlap in DNA damage response and immune surveillance pathways. Utilizing large annotated microarray datasets from normal controls and MM patients, we determined that more than half of the additive genes were differentially expressed between patients and controls (p<0.05). We have confirmed the expression of the most affected gene and have evidence to support its down-regulation as an essential component of the synergy. Our data demonstrate the preclinical efficacy of simultaneously inhibiting HDACs and MTOR, and provide insight into clinically relevant mechanisms in the synergy of the combination.

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Molecular Basis and Modification of a Neural Crest Deficit in a Down syndrome Mouse Model. Samantha L. Deitz. Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN.
Trisomy 21 occurs in ~1/700 live births and leads to phenotypes associated with Down syndrome (DS), including craniofacial dysmoprhology and a small mandible. Ts65Dn mice are trisomic for approximately half the genes on human chromosome 21 and display DS-like craniofacial anomalies. We traced the origin of the small mandible in Ts65Dn mice to a reduced 1st branchial arch (BA1) and deficits in neural crest (NC) migration from the neural tube (NT) and BA1 proliferation around embryonic day 9.5 (E9.5). At E13.5, the small trisomic mandibular precursor persists in Ts65Dn embryos. *DYRK1A* and *RCAN1* are thought to be involved in DS craniofacial development and we hypothesize that dysregulation of *Dyrk1a* and *Rcan1* contributes to the altered craniofacial development in Ts65Dn mice. To test our hypothesis, we quantified expression of *Dyrk1a* and *Rcan1* during embryogenesis. At E9.5, *Dykr1a* is upregulated and *Rcan1* downregulated in Ts65Dn as compared to euploid BA1, yet in the Ts65Dn E13.5 mandibular precursor, *Rcan1* is upregulated and *Dyrk1a* is downregulated. Cells cultured from Ts65Dn and normal BA1 and NT were used to analyze the effects of genetic dysregulation on cell proliferation and migration. In vitro studies revealed a proliferation deficit in trisomic BA1, but not NT, which could be attenuated with the green tea polyphenol epigallocatechin gallate (EGCG, known to modulate the effects of *Dyrk1a*). Additionally we are assessing the effect of EGCG on migration deficits in trisomic NC. Our results provide information about the molecular basis of DS craniofacial abnormalities and may lead to evidenced-based therapeutic options.

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Sequence variation amongst 17 classical and wild-derived mouse genomes and its affect on gene regulation and phenotypic variation. David J. Adams[1], Thomas Keane[1], Leo Goodstadt[2], Petr Danecek[1], Bret Payseur[3], Michael White[3], Kim Wong[1], Binnaz Yalcin[2], Andreas Heger[4], Avi Agam[2], Christoffer Nellaker[4], Martin Goodson[2], Nick Furotte[5], Eleazar Eskin[5], Ian Jackson[6], Richard Mott[2], Laura Reinholdt[7], Chris Ponting[4], Ewan Birney[8], Jonathan Flint[2]. 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/HlLtJ, NOD/ShiLtJ, CAST/EiJ, PWK/PhJ, WSB/EiJ, 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 quantitative trait loci (QTLs), and show that the molecular nature of functional variants and their position relative to genes varies according to the effect size of the locus. Following on from the sequencing of these genomes we will also report on our progress in generating complete de novo assemblies of each strain and the use of the mouse genomes sequence in conjunction with whole exome sequencing.

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The Collaborative Cross: Genetic Architecture of the Combined US and Israel Populations. Fernando Pardo-Manuel de Villena. Genetics, Univ of North Carolina at Chapel Hill, Chapel Hill, NC.

The Collaborative Cross (CC) is a large panel of multiparental recombinant inbred mouse lines specifically designed to overcome the limitations of existing mouse genetic resources. In the past year several reports have described experiments that use emerging CC lines to study a wide variety of phenotypes ranging from voluntary exercise to susceptibility to infectious agents. Although these reports clearly demonstrate the value of the CC, they did not attempt to combine the two main CC populations (from US and Israel) nor did they provide a comprehensive view of the genome of CC lines that are most likely to survive the inbreeding process. To overcome these limitations we have genotyped a single sample from each extant line from both populations using the Mouse Universal Genotyping Array (MUGA) array. We assigned each region of the genome to the appropriate combination of founders. We determined the founder contribution in each sample to identify lines with breeding errors and architecture of the overall population. We used our recent determination of the haplotype diversity and subspecific origin in laboratory strains to predict these features in the completed CC lines. We also compare the performance of the CC with other popular mouse reference populations with regard to the level and distribution of genetic diversity captured, population structure and mapping resolution. These analyses demonstrate the superiority of the CC compared to classical recombinant inbred and consomic lines and panels of selected inbred lines. Finally, we will present our plans and initial analysis of whole genome sequencing of completed CC lines.

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Genotyping Resources for the Laboratory Mouse. Gary A. Churchill. Jackson Lab, Bar Harbor, ME.

Genome-wide association studies in human populations have raised the bar for genetic mapping and analysis of Mendelian and complex traits. However, the genetic structure of human populations, uncontrolled environmental variables, and limitations on experimental interventions and phenotyping present significant barriers to investigations of biological processes in humans. If we could design an ideal model system for genetic studies, what properties would it have? High genetic diversity is desirable to enable the broadest possible scope of discovery. High mapping resolution is needed to identify causal genes with confidence and precision. Absence of population structure and rare alleles combined with full genomic sequences of ancestral haplotypes will substantially improve power and reduce required sample sizes. A good model system does not need to have a natural population structure but the evolutionary origin and context in which segregating variants arose should be understood. The ability to reproduce genotypes leads us to consider inbred models, but natural heterozygosity is also desirable. In depth phenotyping tools, a high-density genotyping platform, and methods to work with transgenic constructs are essential. An experimental system should allow for both discovery and validation. I will describe how the Collaborative Cross and Diversity Outbred mouse populations together fulfill these criteria and provide an ideal system for genetic analysis in a mammalian model organism.

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THE INTERNATIONAL MOUSE PHENOTYPING CONSORTIUM - FUNCTIONAL ANNOTATION OF THE MOUSE GENOME. Steve D. Brown, International Mouse Phenotyping Consortium. MRC Harwell, United Kingdom.

Following the completion of the mouse genome sequence, the International Knockout Mouse Consortium is generating conditional mutants for all mouse genes. An even greater challenge will be the determination of phenotypic outcomes for each mutation. The International Mouse Phenotyping Consortium (IMPC) will undertake the development of an Encyclopedia of the Mouse Genome, building on the several pilot programmes already underway which are exploring the feasibility of large-scale mouse phenotyping. One programme, EUMODIC, is undertaking broad based primary phenotyping of 500 mutants from the EUCOMM programme. The phenotyping pipeline incorporates 20 phenotyping platforms measuring 406 phenotype parameters and 155 metadata parameters. 500 mutant lines have now been committed to the phenotyping pipelines, and data for over 300 lines has been entered into the EuroPhenome database. Of the lines analysed to date, around 60% demonstrate a phenotype at high significance level ($p<0.0001$). Moreover, the analyses reveal a high degree of pleiotropy with most annotated lines showing more than one phenotype annotation. Ontological terms have been assigned and an analysis of the distribution of phenotype annotations according to the ontological terms indicates that a broad spectrum of disease phenotypes has been uncovered. The IMPC incorporates many of the major mouse centres around the world. The IMPC envisages two phases to its programme: Phase 1, 2011-2016 which will undertake the phenotyping of at least 4000 mouse lines; and Phase 2 from 2016-2021 which will undertake the analysis of 15,000 mouse lines. IMPC centres will operate a core, standardised, broad based phenotyping pipeline encompassing the major biological and disease systems. Data from each centre will be uploaded to a central Data Coordination Centre, and following QC and analysis will be archived and disseminated to the wider biomedical sciences community. Preparations for Phase 1 are well underway and I will report on progress in phenotyping pipeline development, IT structures and programme launch.

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Deep sequencing of mouse exome for modeling human diseases encompassing gene-to-gene interactions. Yoichi Gondo, Ryutaro Fukumura, Takuya Murata, Shigeru Makino. Mutagenesis & Genomics Team, Riken BioResource Ctr, Tsukuba, Japan.

We have established deep mouse exome sequencing by target enrichment of 49.6-Mb of entire protein-coding sequences in the mouse. The enriched genomic DNA samples were subjected to ultra-deep sequencing with Illumina and/or SOLiD. This system successfully revealed heterozygous base substitutions in the ENU-mutagenized G1 mouse genomes. By targeting 49.6-Mb exome, we have so far founc 54.8 ENU-induced mutations per G1 genome on an average (SD=12.8) with the validation by the conventional sanger sequencing from the many candidate signals. We have been providing mutant mouse strains carrying an ENU-induced mutation in any target gene to the research community since 2002. The established mutant strains are usually subjected to make it congenic prior to the phenotype analysis, since each G1 mouse carries thousands of ENU-induced mutations in addition to the one in the target gene. Thus, it takes years of backcrosses. At the same time any modifiers or epistatic mutations are discarded on purpose. The newly established mouse exome sequencing should provide a good tool to conduct the phenotype analysis without the years of backcrosses. The G3 phenotype(s) focusing on the mutation in the target gene without backcrosses may be affected by another ENU-induced mutation(s). The genotyping of >50 genomewide mutations identified by the deep exome sequencing should reveal such gene-to-gene interactions if any. The modifier(s) may well be existed in the coding sequences; thus, it is plausible to be directly identified by the exome sequencing. Even if the exome sequencing overlooked the critical mutation(s) or the modifier(s) existed in noncoding sequence(s), the nearby exome mutation(s) should give rise to a good marker(s) to conduce quick-and-easy QTL mapping, since only a few ENU-induced mutations distributed every cM on an average.

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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[2], Nikki Walter[1,2], Priscila Darakjian[2], Sunita Kawane[2], Xusheng Wang[3], Robert P. Searles[2], Robert W. Williams[3], Kari J. Buck[1,2], Shannon K. McWeeney[2], Robert Hitzemann[1,2]. 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 publically 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 4 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 MISO (Katz et al. 2010) to look for differentially expressed isoforms between the 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.

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TNNI3K 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.

TNNI3K is a cardiac specific kinase whose biological function remains largely unknown. We have recently shown that TNNI3K 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 TNNI3K protein and its kinase activity. Firstly, using antisera to mouse and human TNNI3K, we show that TNNI3K localizes at the sarcomere Z disc, primarily inside the desmin ring surrounding the disc; our data also suggest that TNNI3K 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 TNNI3K is a dual-function kinase with Tyr and Ser/Thr kinase activity. To further investigate TNNI3K kinase activity in vivo, we have generated transgenic mice expressing both wild-type and kinase-dead versions of the human TNNI3K protein. Importantly, we show that the increased TNNI3K kinase activity induces mouse cardiac hypertrophy, and the kinase activity is required to accelerate disease progression in a left-ventricular pressure overload model of mouse cardiomyopathy. We demonstrate the clinical relevance of these observations by identifying two potential missense mutations near the kinase activation loop of TNNI3K in idiopathic dilated cardiomyopathy (DCM) human patients. These combined data indicate that TNNI3K is a sarcomeric Z disc kinase that mediates cytoplasmic signaling to sarcomeric structural proteins to modulate cardiac response to stress. TNNI3K may play an important role in cardiomyopathy, and has strong potential as a pharmaceutical target of kinase inhibitors for heart disease.

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Genetic modifiers of *Tgfb1* knockout mice modulate severity in the human vascular disease, HHT. R. Akhurst[1], F. Clermont[1], T. Letteboer[1,2], M. Benzinou[1], K. Harradine[1], R. Roy[1], B. Aouizerat[1], J. K. Ploos van Amstel[2], S. Giraud[3], S. Dupuis-Girod[3], G. Lesca[3], H. Plauchu[3], C. Westermann[2]. 1) HDFCCC, UCSF, San Francisco, CA; 2) Department of Medical Genetics, UMC, Utrecht, The Netherlands; 3) Service de Génétique Moléculaire et Clinique, Hôpital Edouard Herriot, Université de Lyon, Lyon, France.

TGFB1plays a central role in many diseases, including cancer, cardiovascular disease, autoimmunity and fibrosis. To interrogate biological mechanisms that regulate TGFB1 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 *Tgfbm3*.^{C57} we found a 1.2Mb region of 129 genomic DNA at another modifier locus, *Tgfbm2*, on mouse Chromosome 1. This 129 genomic "contaminant" at *Tgfbm2* was present despite multiple generations of backcrossing to NIH. We assume that this 129 variant had been co-selected in *trans* with the *Tgfb1*^{KO} allele. *Tgfbm2*^{129} 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 TGFβ spectrum vasculopathy, Hereditary Hemorrhagic Telangiectasia (HHT), demonstrated that one of the five genes within syntenic *TGFBM2* associates with disease severity ($P= 2 X 10^{-5}$) , 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 to PAVM in HHT was seen within *TGFBM2*, 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.

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Loss of the BMP antagonist, SMOC-1, causes Ophthalmo-Acromelic (Waardenburg Anophthalmia) Syndrome in Humans and Mice. Joe Rainger[1], Ellen van Beusekom[2], Lisa McKie[1], Jacqueline Ramsay[1], Ian J. Jackson[1], Han Brunner[2], Dagmar Wieczorek[3], Hans van Bokhoven[2], David R. FitzPatrick[1]. 1) Medical & Developmental Genetics, IGMM MRC Human 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.

Ophthalmo-acromelic syndrome (OAS), also known as Waardenburg Anophthalmia syndrome, is defined by the combination of eye malformations, most commonly bilateral anophthalmia, with post-axial oligosyndactyly. Homozygosity mapping and subsequent targeted mutation analysis of a locus on 14q24.2 identified homozygous mutations in *SMOC1* (SPARC-related modular calcium binding 1) in eight unrelated families. Four of these mutations are nonsense, two frame-shift and two missense. The missense mutations are both in the second Thyroglobulin Type 1 (Tg1) domain of the protein. The orthologous gene in the mouse, *Smoc1*, shows site- and stage-specific expression during eye, limb, craniofacial and somite development. We also report a targeted pre-conditional gene-trap mutation of *Smoc1* that reduces mRNA to ~10% of wild-type levels. This gene-trap results in highly penetrant hindlimb post-axial oligosyndactyly in homozygous mutant animals (*Smoc1*$^{-/-}$). Eye malformations, most commonly coloboma, and cleft palate occur in a significant proportion of *Smoc1*$^{-/-}$ embryos and pups. Thus partial loss of Smoc-1 results in a convincing phenocopy of the human disease. SMOC-1 is one of the two mammalian paralogs of *Drosophila* Pentagone, an inhibitor of decapentaplegic. The orthologous gene in *Xenopus lævus*, *Smoc-1*, also functions as a BMP antagonist in early embryogenesis. We provide evidence that loss of BMP antagonism during mammalian development provides a plausible explanation for both the limb and eye phenotype observed in humans and mice.

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Downregulation of Hedgehog Signaling Prevents Renal Cystogenesis in Mouse Models of Ciliopathies. David R. Beier, Pamela V. Tran. Genetics/Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, MA.

Cystic kidney disease (CKD), a leading cause of renal failure, is proposed to originate from an underlying ciliary defect. There are no proven therapies for CKD and molecular mechanisms remain unclear. In our characterization of the ENU mutant mouse alien (*aln*), we identified a novel ciliary protein, THM1 (also called IFT139 and TTC21B), which negatively regulates Hedgehog (Hh) signaling. *IFT139* has recently been found to be the most commonly mutated gene in ciliopathies that feature renal cysts as a major clinical component, including nephronophthisis, Bardet-Biedl Syndrome and Meckel-Gruber Syndrome. This prompted us to examine the kidney phenotype in *aln*. By E16.5, *aln* develops renal cysts in the proximal tubules. Importantly, these were markedly reduced in *aln, Gli2-/-* double mutant mice, demonstrating that removal of *Gli2*, the main transcriptional 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 by the small molecule Hh 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 other models of CKD, such as the *jck* mutant, a mouse model for the human cystic 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 protective 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.

Platform Session 2

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

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Functional genomics in mice by whole exome sequencing. Laura Reinholdt[1], David Bergstrom[1], Steve Murray[1], Michelle Curtain[1], Heather Fairfield[1], Carol Bult[1], Joel Richardson[1], Lucy Rowe[1], Mary Barter[1], Daniel Gerhardt[2], Mark D'Ascenzo[2], Todd Richmond[2], Tom Albert[2], Jeffrey Jeddeloh[2], Mona Spector[3], Sylvia He[3], Scott Lowe[3], Jay Shendure[4], Leah Rae Donahue[1]. 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 C57BL6(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 ratio, overlap with RefSeq / 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.

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Pleiades and CanEuCre: MiniPromoters, Cre/ERT2-Driver Mice, and Cre Adeno-Associated Viruses Designed for Selected Expression in the Brain, Eye, and Spinal Cord. EM Simpson[1,2,3], C. de Leeuw[1,2], KG Banks[1], S. Laprise[1], J.-F. Schmouth[1,2], RJ Bonaguro[1], A. McLeod[1], L. Dreolini[4], DJ Swanson[1], L. Liu[5], E. Portales-Casamar[1], MI Swanson[1], SJM Jones[2,4,6], RA Holt[3,4,6], WW Wasserman[1,2], D. Goldowitz[1,2]. 1) Centre Mol Med & Therapeut, UBC, Vancouver, BC, Canada; 2) Dept Med Gen, UBC, Vancouver, BC, Canada; 3) Dept of Psych, UBC, Vancouver, BC, Canada; 4) Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada; 5) Dept.of Anat & Neurobiology, U. of Tennessee, Memphis, TN, USA; 6) Dept Mol Biol and Biochem, SFU, Burnaby, BC, Canada.

The Pleiades Promoter Project (www.Pleaides.org) is a large-scale functional-genomics endeavour focused on generating novel MiniPromoters to drive selected expression in the brain, eye, and spinal cord. This project integrates genome-wide bioinformatics, *in vivo* mouse testing, and histological examination of expression, to develop human MiniPromoters of 4 kb or less. Using this approach we have already developed 27 novel brain MiniPromoters (Portales-Casamar *et al*., PNAS, 2010). We now present an expansion of that collection, adding novel MiniPromoters with unique expression patterns. Of greatest interest may be the sub-regional promoters for the thalamus. Overall, 33% of the MiniPromoters tested expressed in the brain, and 50% of those showed expression related to the gene from which they were designed.

Building on Pleiades, we have launched a new genomics initiative called CanEuCre. This project will develop cre/ERT2-driver mice and cre adeno-associated viruses (AAV) for selected expression in the brain, eye, and spinal cord. As with Pleiades, the cre-driver mice will employ MiniPromoters and MaxiPromoters (BACs) knocked-in single-copy at the *Hprt* locus. The promoters will drive inducible cre/ERT2, and after homologous recombination in ESCs, 35 new mouse strains will be derived. In addition, we will develop and test 50 MiniPromoters directly in AAV in the mouse brain. Most importantly, we are offering opportunities for researchers to nominate new genes or targets for design of MiniPromoters, cre/ERT2-driver strains, or AAV.

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Mouse *Gentrepid*, a webserver tool for candidate gene prediction in mice and humans. Sara Ballouz[1,2], Jason Liu[1], Martin Oti[3], Bruno Gaeta[2], Duncan Sparrow[4], Sally Dunwoodie[4], Merridee Wouters[5]. 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.

Candidate 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.

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KOMPCluster: A Pattern Recognition and 3D Visualization System for Phenotyping Projects. Eric K. Engelhard[1], Kent Lloyd[1], David West[1,2]. 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 inference of gene function by associated phenotypes. To date, the bulk of these results have been presented as gene-centric annotation pages and simple list associations. Unfortunately, this type of data presentation requires that researchers study an ever increasing volume of data in order to discover genotype-phenotype relationships. Here we present KOMPCluster, an alternative data analysis and presentation system for phenotyping projects. KOMPCluster uses unbiased pattern recognition methods to discover and visualize relationships within phenotyping data collections. These patterns are then promoted as starting points to prompt knowledge-driven discovery. In one specific example, Set Theory was used to establish a distance matrix among phenotyping data for ~500 knock out lines provided by a collaboration between Lexicon Pharmaceuticals and Genentech. The distance matrix was further processed with a nearest-neighbor algorithm, which assembled tree graphs across the entire data set. The resulting tree graph was probed with a 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 (Sulf2, Tmprss4, and Slc44a3) among five members ($p = 0.0013$). Interestingly, a fourth member of the same cluster (Hhipl2) 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 behavioral phenotype exceptions. Supported by NIH Grants: 3U01HG004080-04S1 and 3U42RR024244-03S2.

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Identifying sample mix-ups in eQTL data. Karl W. Broman[1], Mark P. Keller[2], Aimee Teo Broman[1], Danielle M. Greenawalt[3], Christina M. Kendziorski[1], Eric E. Schadt[4], Brian S. Yandell[5,6], Alan D. Attie[2]. 1) Department of Biostatistics and Medical Informatics, University of Wisconsin-Madison; 2) Department of Biochemistry, University of Wisconsin-Madison; 3) Merck & Co., Inc; 4) Pacific Biosciences of California; 5) Department of Statistics, University of Wisconsin-Madison; 6) Department of Horticulture, University of Wisconsin-Madison.

In a mouse intercross, with more than 500 animals and genome-wide gene expression data on six tissues, we identified a high proportion of sample mix-ups in the genotype data, on the order of 15%. Local eQTL with extremely large effect (particularly the artifactual eQTL that result from polymorphisms within array probes) may be used to form a classifier for predicting an individual's eQTL genotype from its gene expression value. By considering multiple eQTL and their related transcripts, we identified numerous 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 genotypes did match the predicted eQTL genotypes. The concordance of predictions across six tissues indicated that the problem was due to mix-ups in the genotypes. 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 DK066369 (to ADA).

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Mapping vQTL: detecting major genetic loci controlling phenotypic variability in experimental crosses. William Valdar[1], Lars Rönnegård[2]. 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.

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Genetic Effects at Pleiotropic Loci are Context-Dependent with Consequences for the Maintenance of Genetic Variation in Populations. Heather A. Lawson[1], Janet E. Cady[1], Charlyn Partridge[1], Jason B. Wolf[2], Clay F. Semenkovich[1], James M. Cheverud[1]. 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.

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A new way to find novel antimalarial drugs using a large-scale ENU dominant screen. Gaetan R. Burgio, Shelley Lampkin, Preethi Mayura-Guru, Fleur Rodda, Elinor Hortle, Andreas Greth, Patrick Lelliott, Brendan McMorran, Simon Foote. Menzies Research Institute, University of Tasmania, Hobart, Australia.

Malaria is any infectious disease of the blood caused by the protozoan parasite, Plasmodium, and is the third most lethal infectious disease in the world. Of major concern, our armamentarium of antimalarial drugs is failing with the increasing frequency of drug-resistant parasite strains. In contrast, there are several examples of human genetic polymorphisms that protect against the disease, and have likely done so for many hundreds of years. Given the selective pressures malaria has exerted on human populations, other host protective mutations have likely arisen, but they remain challenging to elucidate. We are interested in identifying such mutations that can be mimicked by drugs, and develop a so-called 'host-directed therapys' (HDT) that avoid parasite drug resistance. Here we have employed a large-scale ENU screen in mice using a rodent model of malaria infection to find mutations that increase resistance to infection. A dominant screen was performed on SJL/J, which is highly susceptible to the rodent malaria. Over 7000 G1s have been challenged, and 35 survival G1s have been identified and confirmed as heritable through progeny testing. To date, using the "affected only" mapping strategy on a C3H/He x ([C3H/He x SJL] F1) crosses, chromosomal locations of the mutation in five of these resistant lines have been mapped, and for three, the mutation identified, one using next generation sequencing technologies and two using candidate gene approach (Ank-1^{MRI23420} and beta1-Spectrin^{MRI26194}). 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.

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The AcB61 and AcB60 recombinant congenic strains of inbred mice: susceptibility and resistance to a mouse model of human typhoid fever. Sean Beatty[1,2], Marie-France Roy[4], JC Loredo-Osti[5], Danielle Malo[1,2,3]. 1) Complex Traits Group; 2) Dept of Human Genetics and; 3) Dept of Medicine, McGill University, Montreal, QC; 4) Dept of Veterinary Clinical and Diagnostic Sciences, University of Calgary, Calgary, AB; 5) Dept of Mathematics and Statistics, Memorial University, St. John's, NFLD.

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 *Nramp1*), 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 129S6. *Ity8* on Chr15 was validated using an (AcB60 x DBA/2J)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.

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Systems genetic analysis reveals a complex and dynamic transcription network governing sex determination. Steven Munger[1], Anirudh Natarajan[1], Loren Looger[2], Blanche Capel[1]. 1) Department of Cell Biology, Duke University Medical Center, Durham, NC; 2) HHMI Janelia Farm, Ashburn, VA.

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 trancription 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 eQTGs 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.

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Identifying suppressors of Mecp2 symptoms in the mouse: a genetic approach to therapeutic intervention. Monica J. Justice[1], Christie M. Buchovecky[1], Frank J. Probst[1], Hannah M. Brown[1], Jill Crowe[1], Jay Shendure[2]. 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 genic 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.

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Genetics and genomics of tumorigenesis in the Chaos3 breast cancer mouse model. John C. Schimenti, Marsha Wallace, Chen-Hua Chuang, Lishuang Shen. Dept Biomedical Sci, Cornell Univ, Col Vet Med, Ithaca, NY.

The *Mcm4*^{Chaos3} mouse model contains the only endogenous gene mutation known to lead exclusively to mammary tumors. *Chaos3* is a point mutation of a highly conserved residue in the minichromosome maintenance 4 (*Mcm4*) gene, detected in a screen for Genomic Instability (GIN). MCM4 is a subunit of the MCM2-7 replicative helicase and is required for DNA replication licensing, which restricts replication to only once per cell cycle. Nearly all homozygous nulliparous females develop exclusively mammary tumors in the C3H background, but the mice develop histiocytic sarcomas and lymphomas in the B6 background. We have taken genomic and genetic strategies to determine the molecular bases for tumor etiology and cancer type susceptibility. Exome resequencing and B6 x C3H F2 crosses point to talin 1 as a potential breast cancer susceptibility modifier. aCGH studies of *Chaos3* mammary tumors revealed that virtually all shared certain amplified or deleted regions, the most notable of which contains the tumor suppressor NF1. Finally, we generated and analyzed the phenotypes of mice that bear combinations of null and *Chaos3* mutations in *Mcm* 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. These studies reveal the necessity for precise control of DNA replication in development and disease prevention, and that *Chaos3* mice are a unique and powerful model to investigate the genetics of cancer susceptibility.

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Ionizing radiation induced DNA strand break (DSB) repair and lymphohematopoietic disease in vivo and in vitro. John E. French[1], Vandy Parron[1], Michael A. Streicker[2], Grace E. Kissling[1], Susan Borghoff[3], Todd Painter[2], Amy E. Brix[3], David E. Malarkey[1]. 1) NIEHS, NIH, Research Triangle Park, NC; 2) Integrated Laboratory Systems, Research Triangle Park, NC; 3) Experimental Pathology Laboratories, Research Triangle Park, NC.

Coordination of DNA strand break (DSB) repair pathways is dependent on the phase of the cell cycle, DNA replication, and p53 tumor suppressor protein expression. p53 protein haploinsufficiency increases error prone repair. We have observed differences in the loss of heterozygosity (LOH) of the wildtype *Trp53* allele, non-random loss of Chr 11 simple sequence length polymorphisms, and the prevalence in ionizing radiation (IR) induced disease in B6 and D2 mice. To determine the genetic basis for this observation we used primary cultures of hematopoietic stem cells (HSC) from B6 and D2 mice and observed strain related differences in the abundance of DNA repair gene transcripts and biomarkers of DSB repair (time related resolution of γH2AX foci). Based on allelic variation in the non-homologous end-joining repair pathway for DSB (associated with LOH), we selected females from 6 strains (129S1.SvlmJ, BTBR.T/J, DBA2/J, BALB/cByJ, and A/J) that represented the maximum genetic diversity in NTP-Perlegen sequenced inbred laboratory derived strains for outcross to B6.129-*Trp53*^{tm1Brd} N13 homozygous null males to produce 6 NHEJ pathway genetically diverse F1 strains. Analysis of each NHEJ DSB repair gene, individually and collectively, by similarity matrices indicates that haplotype diversity between isogenic strains is significant, but a number of genes in the DSB repair pathways are identical by descent. Twelve male and 12 female F1 progeny of each outcross were exposed to 0, 3, or 6-Gy ionizing radiation and observed for 40 wks post-exposure. We observed significant differences in strain-related latency (time to 50% tumor bearing mice), survival, and tumor phenotypes (lymphoma and leukemia) and penetrance. We speculate that the fidelity of radiation induced DSB repair associated with LOH and tumor susceptibility are complex polygenic traits.

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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 interindividual variation in cancer susceptibility to carcinogen-induced epithelial cancers. We have mapped several loci that modify susceptibility to skin tumor promotion using crosses 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 underlying a complex locus, *Psl1*, on mouse Chromosome 9. We also showed that susceptibility to nonmelanoma skin cancer in humans is associated with inheritance of polymorphisms in *GSTA4*. The goal of this study is to identify promotion susceptibility genes that modify the response to divergent classes of skin tumor promoting agents. To accomplish this, we are conducting skin tumor experiments using a panel of BxD recombinant inbred (RI) strains. Mice from each strain were initiated with 7,12-dimethylbenz(a)anthracene and promoted with by 12-O-tetradecanoylphorbol-13-acetate (TPA) or chrysarobin. As expected, tumor response varied from nonresponsive strains, similar to C57BL/6, to hyper-responsive strains developing significantly more tumors at an earlier age than observed for DBA/2 mice. Analysis of tumor response of BxD RI strains promoted with TPA suggested that a promotion susceptibility locus maps to Chromosome 13, consistent with previous studies using a different panel of BxD RI strains initiated with N-methyl-N'-nitro-N-nitrosoguanidine and promoted with TPA. When combined, these data revealed a significant linkage mapping to a 12 Mb region of distal Chromosome 13 with several genes having amino acid variants which may be candidate promotion susceptibility genes.

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Pharmacological correction of cerebellar dysmorphology has a surprising salutary effect on hippocampal-based learning and memory tasks in the Ts65Dn mouse model of Down Syndrome. Roger H. Reeves[1,2], Ishita Das[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 human 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 hypocellular. We traced the cerebellar hypocellularity 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 in hippocampus are characteristic of people with DS and are also impaired in Ts65Dn mice. Trisomic mice have a robust deficit in the Morris Water Maze (MWM), a visiospatial task classically associated with hippocampus. Three month old trisomic mice that had received a single injection of SAG at P0 performed as well as their euploid littermates in this and other hippocampal-based tasks. We found no structural normalization of hippocampus in SAG-treated mice, although cerebellar morphology was identical to euploid. Summary: A single exposure to a small molecule agonist on the day of birth completely restores cerebellar morphology and has a surprising salutary effect on hippocampal function in adult mice that model structural and behavioral aspects of Down syndrome.

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Idiosyncratic adverse drug reactions modeled using a genetically diverse mouse panel may facilitate pharmacogenomics. Alison H. Harrill[1], Karissa Adkins[2], Hong Wu[2], Mathew T. Pletcher[2], Paul B. Watkins[1]. 1) The Hamner Institutes; 2) Pfizer, Inc.

Drug-induced liver injury (DILI) is the major adverse drug event that leads to regulatory actions on drugs. The most problematic and poorly predictable form of DILI is "idiosyncratic," meaning the drug is safe for the vast majority of treated patients while causing liver injury in a few susceptible individuals, suggesting a possible genetic contribution. We previously demonstrated that a Mouse Diversity Panel (MDP) can be utilized to mimic responses of human cohorts to liver toxicity induced by acetaminophen, a dose-dependent hepatotoxic drug, and to detect a genetic risk factor for human liver injury susceptibility. To test the ability of an MDP to predict clinical toxicities that are not observed in classical preclinical models, we are engaged in a broad industry collaboration coordinated through the Health and Environmental Sciences Institute (HESI). Isoniazid, an anti-tuberculosis drug 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 INH DILI. Thirty-four genetically diverse mouse strains comprising the MDP were treated with isoniazid (100 mg/kg, i.g.) or vehicle once daily for three days (N=4 per strain and treatment). We observed a strain-dependent increase in incidence and severity of microvesicular steatosis (42% of strains) and in liver triglyceride levels (15% of strains), consistent with mitochondrial toxicity that may predispose individuals to overt hepatotoxicity. Liver transcriptome and serum cytokine profiling is underway. Quantitative trait loci mapping of steatosis and triglyceride levels will inform interrogation of the DILIN whole exome clinical data for candidate susceptibility loci. Our data support the idea that the mouse population-based approach may have utility to predict clinical toxicities where other animal models have failed. A mouse population-based approach provides an attractive model for risk assessment of new drugs and detection of pharmacogenetics marker identification.

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Genetic variation in expression of renal Folr1 (folate receptor 1) as a risk factor for features of the metabolic syndrome. Michal Pravenec[1], Viktor Kozich[2], Ludmila Kazdova[3], Theodore W. Kurtz[4]. 1) Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic; 2) Institute of Inherited Metabolic Disorders, 1st Medical Faculty, Charles University, Prague, Czech Republic; 3) Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 4) University of California, San Francisco, CA.

Metabolism of homocysteine and other sulfur amino acids is closely associated with metabolism of folates. In the current study, we analyzed the possible role of folates/sulfur amino acids in the development of features of the metabolic syndrome in the spontaneously hypertensive rat (SHR). First, we demonstrated that the SHR strain compared to the normotensive Brown Norway (BN-Lx) strain exhibits significantly lower plasma folate concentration and increased plasma total homocysteine and cysteine. Next, in BXH/HXB recombinant inbred (RI) strains derived from SHR and BN-Lx progenitors, we mapped a QTL for cysteine concentrations to a region of Chromosome 1 that contains a cis-acting expression QTL regulating mRNA levels of *Folr1* (folate receptor 1) in the kidney. Sequence analysis revealed a 5.7 kb insertion/deletion variant in the *Folr1* promoter region of the the BN versus SHR strains. Results in the SHR.BN-chr.1 congenic strain with the BN *Folr1* allele showed that the SHR variant in *Folr1* cosegregates with reduced renal expression of *Folr1*, reduced renal folate reabsorption, decreased serum levels of folate, increased serum levels of cysteine and homocysteine, reduced muscle insulin sensitivity, and increases in blood pressure. Transgenic rescue experiments in the SHR with increased expression of the *Folr1* transgene confirmed these results. These findings are consistent with the hypothesis that inherited variation in expression of *Folr1* inside the kidney affects features of the metabolic syndrome and constitutes a previously unrecognized genetic mechanism that may contribute to the pathogenesis of cardiovascular disease. (supported by grant NS10036-3 from the Ministry of Health of the Czech Republic).

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Mining phenotypes and disease models in MGI. J. T. Eppig, S. Bello, H. Dene, D. Burkhart, H. Onda, R. Babiuk, M. Knowlton, M. Tomczuk, J. Xu, A. Anagnostopoulos, C. L. Smith. Jackson Laboratory, Bar Harbor, ME.

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 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) database is a treasure trove for biologists and clinicians seeking to correlate mouse and human phenotypes. 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 >585,000 alleles are cataloged in >19,000 genes. About 28,000 alleles are propagated in live mice; the rest existing in ES cell lines. The amount of phenotypic data available is already very rich, with nearly 43,000 unique genotypes curated with >203,000 MP terms. This substantial data set makes possible mining of MGI to look for phenotype associations (e.g., What phenotypes are found concordantly [or not]? What genotypes have the same phenotype profile as the one I am interested in?). Such questions can uncover genes in a common pathway, implicate genes with particular functions, or identify relationships between diseases and their underlying defects. User access to mouse phenotype and disease data will be presented and is achieved in various ways from a gene, allele, phenotype, or disease perspective. MGI's infrastructure is ready to accept data from the new International Mouse Phenotyping Consortium (IMPC) Centers, which plan large-scale phenotyping of the collection of knockouts and conditional-ready knockouts of every mouse gene. Stay tuned for new access and analysis of these data! Supported by NIH grant HG000330.

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Next steps in systemic analysis of mouse mutants at the German Mouse Clinic. Martin Hrabé de Angelis, Valérie Gailus-Durner, Helmut Fuchs, GMC Consortium. GMC, Institute of Experimental Genetics, Helmholtz Zentrum München, Munich, Germany.

The next steps in the full exploitation of mutant mouse lines as models for human diseases are the systematic (one after the other) and systemic (assessing all organ systems) phenotyping of mutant mouse resources. Additionally, for the next generation of mouse models the envirotypes that humans are exposed to, need to be modeled. To accomplish these goals we have established the German Mouse Clinic (GMC) (www.mousclinic.de) as phenotyping platform with the logistics of systemic, standardized phenotypic analysis and open access for the scientific community on a collaborative basis.

We have been setting up challenge platforms to explore the complex relationship between environmental changes and genetic factors. By simulating specific and standardized environmental exposures or life styles we mimic envirotypes that have a strong impact on human health such as nutrition, activity, air, infection and stress. Our goal is to decipher their effects on disease etiology and progression, uncovering the physiological and molecular mechanisms of genome-environment interactions.

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 *Nox4* revealed almost no effects of the knockout on the organism. However, these mice were largely protected from oxidative stress, blood-brain-barrier leakage, and neuronal apoptosis after cerebral ischemia. NOX4 therefore represents a novel class of drug target for stroke therapy. Mice harboring a missense mutation in the phospholipase Cγ2 gene (*Plcg2*) display an inflammatory arthritis phenotype. Systemic phenotyping of *Plcg2*^{Ali14} mice uncovered multiple immunological and metabolic changes including an *in vitro* infertility phenotype. This demonstrates the value of the *Ali14/+*; mouse as a model for systemic inflammatory diseases and inflammation-related metabolic changes in humans.

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Effect of genetic and environmental variation on differential DNA methylation in mouse. John D. Calaway[1,3,4], John P. Didion[2,3,4], Ginger D. Shaw[3,4], Jason S. Spence[3,4], Ron Korstanje[5], Gary A. Churchill[5], Fernando Pardo-Manuel de Villena[1,2,3,4]. 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.

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Novel methods of mapping QTL, QTS and QTT for complex traits in mouse. Jun Zhu[1], Zhihong Zhu[1], Chenhao Zhang[1], Xuseng Wang[2], Lu Lu[2], Robert Williams[2]. 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 (h^2=0.27) and 3 pairs of epistasis QTLs (h^2=0.23). By using 2,320 SNPs, 17 QTNs were detected for 7 main-effect QTNs (h^2=0.25) and 6 pairs of epistasis QTNs (h^2=0.22). We used a small mapping population (188 individuals in 5 treatments), and detected only one main QTT (h^2=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 QTSs 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.

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ENU-induced mutation in *Ank1* results in hemolytic anemia and increased susceptibility to *Salmonella* Typhimurium infection. Kyoko E. Yuki[1,3], Megan M. Eva[1,3], Silvia Vidal[1,3], Marilène Paquet[4], Danielle Malo[1,2,3]. 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-nitrosurea (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 uninfected 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.

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Host genetic susceptibility to influenza A infections. Klaus Schughart[1], Esther Wilk[1], Tatiana Nedelko[1], Heike Kollmus[1], Xavier Montagutelli[2], Claudia Pommerenke[1]. 1) Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Lower Saxony, Germany; 2) Mouse Functional Genetics, 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 BcG 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.

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Collaborative Cross mice and their power to map host susceptibility to *Aspergillus fumigatus* infection. Caroline Durrant[1], Hanna Tayem[2], Binnaz Yalcin[1], James Cleak[1], Leo Goodstadt[1], Fernando Pardo-Manuel de Villena[3], Richard Mott[1], Fuad A. Iraqi[2]. 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 homozygous 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 1.18Mb. 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.

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***Prdm14* alters gene expression in an expanded common lymphoid progenitor-like population prior to initiating leukemia.** Stephen J. Simko[1], EJ Dettman[2], Bernard A. Ayanga[2], Brandi L. Carofino[2], Judith F. Margolin[1], Monica J. Justice[2,3]. 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 marrow 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 murine stem cell virus-based vector containing *Prdm14* or empty vector. Transduced marrow was transplanted into lethally irradiated mice. Prior to onset of leukemia, marrow cell suspensions underwent cell sorting for multiple hematopoietic progenitor lines, including common lymphoid progenitors (CLPs; $Il7ra^{+}lin^{-}Kit^{+}Sca1^{+}$). Gene expression arrays were performed on $Il7ra^{+}lin^{-}Kit^{+}Sca1^{+}$ cells from experimental and control mice. MicroRNA expression in mature tumors was also evaluated. $Il7ra^{+}lin^{-}Kit^{+}Sca1^{+}$ 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 signal transduction were downregulated. In our tumors, these expression changes were recapitulated, and additional pluripotency genes and oncogenes were overexpressed, including *Prdm1*, *Pou5f1*, and *Myb*. 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.

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Recognition of maternal signature odor mixtures mediates the first suckling episode in mice. Darren W. Logan[1,5], Lisa J. Brunet[2], William R. Webb[3], Tyler Cutforth[4], John Ngai[2], Lisa Stowers[5]. 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 dehydration 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 neonate 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 malleable 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.

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Systems Genetics of Male Infertility in Extinct Collaborative Cross Lines. David L. Aylor[1], Timothy A. Bell[1], Ryan J. Buus[1], Mark E. Calaway[1], Darla R. Miller[1], Fanny Odet[2], Jenny Yu[1], Deborah A. O'Brien[2], Fernando Pardo-Manuel de Villena[1]. 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 *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.

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Functional annotation of autism genes using genomic resources in the mouse. M. Bucan[1,2], K.-J. Won[1], L. Wang[2], E. Clevac[2], B. Georgi[2], S. Wadhawan[1]. 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://sfari.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.

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An ENU mouse model of a GWAS obesity gene - a neuronal-specific role in energy homeostasis. Angela W. S. Lee[1], Michael Schaefer[2], Roger D. Cox[1]. 1) 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 regulator1(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 (6-11%, $P<0.01$) due to a reduction in lean mass (7-11%, $P<0.01$) 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 *Pdk4*, suggesting that glycogen 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 ENU mutagenesis to identify the underlying genes.

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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. Epidemiogical 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 α (ERα) deficient mice on the lupus prone (NZB x NZW)F1 background, we have shown that ERα mediates 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.

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Spink3 is involved in pancreatitis through the regulation of autophagy. Ken-ichi Yamamura, Kimi Araki, Masaki Ohmuraya. Insitute of Reource 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 SPINK3deficient 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 conditional 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 antophagy is involved in activation of trypsinogen. Taken together, hereditary pancreatitis in human caused by the mutation of *SPINK1* gene may be due to excessive autophagy induction, but not to loss of trypsin inhibitor activity.

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Novel early homolog pairing mediated by SUN1 and a SPO11 double-strand break independent activity during mammalian meiosis. Kingsley A. Boateng, Marina A. Bellani, Rafael D. Camerini-Otero. Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD.

Genomic stability in eukaryotes depends on the accurate distribution of chromosomes during meiosis. Consequences of a failure to properly segregate meiotic chromosomes in animals range from aneuploidy to infertility. The pairing and alignment of homologous chromosomes in meiosis is arguably the premiere genetic event. It is widely believed, and we have argued (1), that in most organisms from yeast to man, with some notable exceptions such as in flies and worms, this pairing and alignment is mediated by events pursuant to the introduction of double strand breaks involving a search for homology mediated by the homologous recombination biochemical machinery. Here we show that, as first demonstrated in budding yeast (2-4), a significant level of pairing in meiotic S-phase (replicating) spermatocytes, as assessed by fluorescent in situ hybridization to either structurally preserved nuclei or in tissue sections, precedes SPO11 mediated cleavage. This is the first report of such early pairing in mammals. Taking advantage of a knock-in catalytic mutant *Spo11* mouse, we show that this pairing requires SPO11, but is independent of its DSB activity, consistent with a previous report in budding yeast (4). Early pairing also requires SUN1, which is essential for gametogenesis and is involved in telomere attachment to the nuclear membrane (5-6). These early homolog interactions are most likely a critical prerequisite for stable pairing interactions during meiotic prophase 1. References 1. Yancey-Wrona J.E. and Camerini-Otero R.D. (1995). Curr. Biol.5:1149 2. Weiner B.M. and Kleckner N. (1994). Cell 77:977-991. 3. Dilip K.N. et al. (1995). Genetics 141:75-86 4. Cha R.S. et al. (2000). Genes & Development 14:493-503. 5. Ding X. et. al. (2007). Dev Cell 12:863-872. 6. Chi Y. et al. (2009). Development 136:965-973.

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Experimental Mammalian Biology: Verne Chapman and the Molecular Genetic Era. William Dove, James Amos-Landgraf, Linda Clipson, Amy Irving, Alexandra Shedlovsky. McArdle Laboratory, Univ Wisconsin, Madison, WI.

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 Community o From Mt. Desert to North America, Europe, and Asia c/o biology oThe Mammalian Genome Society, the GSA and other communities: their intersections on biological planes.

66B

Mutation of the carbamoyl-phosphate synthetase 2 (*Cad*) leads to embryonic lethality in mouse. Yung-Hao Ching[1,2], Erin K. Stenson[2], John C. Schimenti[2]. 1) National Laboratory Animal Center, Taipei, Taiwan; 2) Center for Vertebrate Genomics, Cornell University, College of Veterinary Medicine T9014A, Ithaca, NY 14853.

Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (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*^{L5Jcs24} and *Cad*^{L5Jcs27}). These are the first two live *Mus musculus* 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*^{L5Jcs24} allele retained carbamoyl phosphate synthetase (CPSase) 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 CPSase activity itself, which is lost in the *Cad*^{L5Jcs27} 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, muscular 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, muscular 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.

68A

Molecular bases of expression of syndromic photoreceptor dystrophies by the RPGR- and cell-type-dependent subcellular sorting, tethering and processing of RPGRIP1α_1. Paulo A. Ferreira, Kyoung-in Cho, Hemangi Patil, Haiqing Yi. Ophthalmology/Pathology, Duke Univ Medical Ctr, Durham, NC.

Human mutations affecting components of the interactome assembled by the Retinitis Pigmentosa GTPase Regulator-Interacting Protein 1 (RPGRIP1) cause severe syndromic retinal dystrophies of variable penetrance and expressivity by elusive mechanisms. The Retinitis Pigmentosa GTPase Regulator (RPGR) interacts with the RPGRIP1 via its RHD domain, which is homologous to RCC1, a nucleotide-exchange factor for RAN GTPase. Molecular modeling of RHD of RPGR to RCC1 shows that all disease mutations in RHD map to a distinct contact interface from that found between RCC1 and RAN GTPase. Two-hybrid assays show that disease-causing mutations in RHD or ORF15 domains differentially impair RPGR interaction with RPGRIP1. Cell-based and time-lapse live-cell microscopy assays support that expression of RPGRIP1α_1 isoform alone promotes profuse intracellular aggregates, whereas its co-expression with the RPGR$_{1\text{-}19}$ isoform targets RPGRIP1α_1 to the Golgi. Conversely, RPGR$_{ORF15}$ co-expression with RPGRIP1α_1 promotes its pan-intracellular dispersion and clears-out pre-existing RPGRIP1α_1 deposits. Co-expression, but not single expression, of disease mutations in RHD of RPGR isoforms and RID of RPGRIP1α_1 abrogates their colocalization and physical association in COS7 cells, whereas mutations singly in any RPGR isoform suffice to promote delocalization from RPGRIP1α_1 in a photoreceptor line. RPGR$_{ORF15}$, but not RPGR$_{1\text{-}19}$, protects RPGRIP1α_1 from limited C-terminal proteolysis. Hence, these studies show that RPGR-dependent and cell type-selective sorting pathways with structural and functional plasticity modulate the expression of mutations in *Rpgr* and *Rpgrip1*. Further, RPGR isoforms are differently necessary to the coupling to, and subcellular sorting of,RPGRIP1α_1, with deficits in RPGR$_{ORF15}$-dependent post-Golgi sorting of RPGRIP1α_1 contributing to pathomechanisms shared by the retinal dystrophies, X-linked retinitis pigmentosa 3 and Leber congenital amaurosis. (NIH GM083165 & EY019492).

69B

Mechanistic insights into regulation of metastasis by Sipa1. Thomas R. Geiger[1], Katie Mattaini[1,2], Mia Williams[1,3], Renard Walker[1], Jude Alsarraj[1], Rosan Nieves Borges[1], Kent Hunter[1]. 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 *Sipa1* as a candidate metastasis gene. Our subsequent analysis demonstrated that Single Nucleotide Polymorphisms (SNPs) in the *Sipa1* gene are associated with metastasis in mice and human breast cancer patients. Consistent with this observation, we have shown that SIPA1 regulates metastasis in breast cancer cells; however, the molecular mechanisms remain largely unknown. We identified several potential binding partners of SIPA1 in a yeast-two-hybrid screen, and confirmed interactions of SIPA1 with BRD4, RRP1B and the inner nuclear membrane protein SUN2 in subsequent experiments. Our analysis suggests that several complexes of SIPA1 exist in different compartments of the cell. A structure-function analysis that we have begun to carry out indicates that SIPA1 regulates metastasis in several ways, depending on its interaction partners and subcellular localization. Furthermore, the enzymatic GTPase-activating function of SIPA1 appears to play a critical role in regulating metastasis. Our results are beginning to reveal a novel mechanism of metastasis regulation, and could facilitate the development of better therapies for the treatment of metastatic breast cancer.

70C

MOUSE HEPATIC LIPASE (*Lipc*): COMPARATIVE GENOMICS, PROTEOMICS AND PHYLOGENY. Roger S. Holmes[1,2], Laura A. Cox[2]. 1) School of Biomolecular 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); HL; 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 lipase genes [*Lipg*: 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 proprotein convertase ('hinge') and heparin binding regions. Predicted secondary and tertiary structures revealed similarities with the 3D structure for horse pancreatic lipase (LIPP). 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 [*LIPG* and *LPL*). We conclude that the triglyceride lipase ancestral gene for the vertebrate *LIPC* gene predated the appearance of fish during vertebrate evolution.

71A

Chromatin Remodeling Complex NURF is a Key Regulator of Chromatin Structure at MHC Class I and Class II Genes. Joseph Landry[1], Suhybe Alkhatib[1], Mark Zimmerman[1], Daniel Murray[1], Carolyn Song[1], Jing Yuan[1], Carl Wu[2]. 1) Virginia Commonwealth University, Department of Human and Molecular Genetics, Virginia Institute of Molecular Medicine, Goodwin Research Labs, 401 College Street, Richmond, VA 23298; 2) National Institutes of Health, National Cancer Institute, Building 37, Room 6068, Bethesda, MD 23892.

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 reestablish 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 H2-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 Oota[1], Yosuke Ikegami[2], Koh Ayusawa[2], Kazuyuki Mekada[1], Nobunori Kakusho[3], Hirotaka Imagawa[2], Hiroyuki Hishida[5], Hiromasa Suzuki[5], Yuichi Obata[1], Hideo Yokota[3], Ryutaro Himeno[4], Yoshihiko Nakamura[2], Atsushi Yoshiki[1]. 1) BRC, RIKEN, Japan; 2) The University of Tokyo, Japan; 3) ASI, RIKEN, Japan; 4) ACCC, RIKEN, Japan; 5) RCAST, 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 anatomy 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*-H2Bcherry:GFPgpi BAC expression to immunohistochemical staining with the widely used PGP9.5 antibody reveals that *Uchl1* is restricted to a discrete subset of neuronal progenitors. This contrasts to immunoreactivity detected wtih PGP9.5 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.

75C

There is more to Solute Carrier Family 11 member 1; (SLC11A1), protein than cytokine regulation. Agnes A. Awomoyi[1], Susan Gauthier[2], Ming-Jie Liu[3], Hee Jung Kim[3], Molly Mo[4], Lianbo Yu[4], Mark D. Wewers[3], William LaFuse[3], Philippe Gros[2]. 1) N Potomac, MD; 2) Department of Biochemistry, McGill University, Montreal Canada; 3) Internal Medicine, Ohio State Medical Center, Ohio State University, Columbu, OH; 4) Center for Biostatistics, 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 MCSF derived macrophages to purified TLR-2 agonist; Pam3CSK4 treatment was determined by comparing microarray gene expression profiles for *Slc11a1*-/- and *Slc11a1*+/+ *BMDMs using the Ilumina mouse WG 6 V 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 </ISLC11A1< i>microarray gene expression profiling analyses, we show that *Slc11a1* regulates p62 levels at basal levels. p62 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. We however, propose that this IL-1B released (whether in exosome vesicles or secreted) should interact with its receptor on adjacent cells, activates the NF-KB 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 known.

76A

High-resolution Mapping of Multiple Complex Traits in Heterogeneous Stock Rats. Amelie Baud, Jonathan Flint, Richard Mott, EURATRANS Consortium. Wellcome Trust Center for Human Genetics, Oxford, United Kingdom.

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, bone 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 r^2 falls below 0.1 within 4Mb. 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 QTLs if the familial structure is ignored. We have compared two approaches to control for familial structure in the HS: resample 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. Carroll[1], Abraham A. Palmer[2,3], David A. Blizard[4], Arimantas Lionikas[1]. 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 Psychiatry and Behavioral Neuroscience, the University of Chicago, IL 60637; 4) The Pennsylvania State University, College of Health and Human Development, University Park, PA 16802.

The functional properties of skeletal muscles are affected by the number, size and type of muscle fibres, all of which vary considerably among individuals. Genetic factors account for a substantial portion of that variation; however, specific mechanisms are poorly understood. We examined soleus fibre properties in two strains of mice divergently selected for large (LG/J) and small (SM/J) body size and their F_2 intercross (n=131). Solei were dissected from 90 day old mice, transversely sectioned and stained for myosin ATPase. A whole genome QTL scan was carried out for fibre number, percentage of type I fibres, and cross-sectional area (CSA) of type I and type IIA fibres. There was no difference in the total number of muscle fibres between the LG/J and SM/J strains (569±95 vs 540±119, respectively) or males and females. A higher percentage of type I fibres was observed in LG/J than SM/J strain ($P<0.001$) in males (45±3 vs 37±4) and females (58±4 vs 41±3). Across strains, females had a higher percentage of type I fibres than males ($P<0.001$), and the sex difference was greater in LG/J strain (strain-by-sex interaction, $P<0.001$). The CSA did not differ between type I and type IIA fibres (1086±385 μm^2 vs 1103±356 μm^2), was greater in the LG/J than SM/J strain (1365±268 μm^2 vs 825±229 μm^2, across fibre type, $P<0.001$), and tended to be greater in males than females (1134±337 μm^2, vs 1055±397 μm^2, $P=0.09$). We found significant QTL for type I and for type IIA CSA mapping to chromosomes 1, 6 and 11. Our results provide the evidence that the LG/SM intercross is a promising model for studying how genetic variation affects muscle fibre properties.

78C

Variation in Body Composition of Genetically Diverse Mice. Gregory W. Carter, Daniel M. Gatti, Gary A. Churchill, Karen L. Svenson. The Jackson Laboratory, Bar Harbor, ME.

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 relationship that is similar to the one found in a human population. 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 regulation of body composition has a significant genetic component and therefore models based on population averages may be unreliable for many individuals.

79A

GENOME ARCHITECTURE IN ISOLATED POPULATIONS OF WILD MICE. John P. Didion[1], Ryan J. Buus[1], Jeremy Searle[2], Fernando Pardo-Manuel de Villena[1]. 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 of acrocentric 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 genome architecture and to investigate the genetic basis of centromeric drive. We found that the frequency of homozygosity (inbreeding) is higher in Rb races than in standard populations, and within Rb races homozygosity is highest at the centromeres. In addition, 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 formation or preferential transmission of Rb translocations and discovered multiple candidates.

80B

Identification of a Transcriptional Network Associated with Osteoblast Activity Using Systems Genetics. Charles Farber[1], Ana Lira[1], Brian Bennett[2], Luz Orozco[2], Hyun Kang[3], Calvin Pan[2], Eleazar Eskin[3], Aldons Lusis[2]. 1) Center for Public Health Genomics, University of Virginia, Charlottesville, VA 22908; 2) Departments of Medicine and Human Genetics, David Geffen School of Medicine at UCLA, UCLA, Los Angeles, CA 90095; 3) Department of Computer Science, UCLA, Los Angeles, CA 90095.

One of the main goals of systems genetics is to unravel the molecular mechanisms of disease by determining how genetic variation perturbs cellular networks. Here, we used systems genetics to identify and characterize gene co-expression networks that influence the *in vivo* activity of bone-forming osteoblasts. To begin a gene co-expression network was generated using bone microarray data from 96 Hybrid Mouse Diversity Panel strains. The resulting network consisted of 10 co-expression gene modules. Of the 10, one module (the purple module or PM) was significantly enriched for genes that were i) preferentially expressed in osteoblasts and ii) known to play critical roles in osteoblast biology. These data suggested that PM genes and their interactions were important for osteoblast activity. To investigate the importance of the PM in an independent model, siRNAs were

used to alter the expression of its most highly connected gene, *Maged1*, in primary calvarial osteoblasts. The reduction in *Maged1* preferentially altered the expression of PM genes (but not genes from other modules), and increased osteoblast proliferation and differentiation. We next identified 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. Swati 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 phenotypic/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. Being able to separate these effects is important for our understanding of the underlying 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 no 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. Iancu[1], Priscila Darakjian[1], Barry Malmanger[1], Jason Erk[1], Denesa Oberbeck[1], Shannon McWeeney[1], Robert Hitzemann[1,2]. 1) Oregon Health & Science University, Portland, OR; 2) Research Service, Veterans Affairs Medical Center, Portland, OR.

We examined the genomic and transcriptional changes induced by short term bi-directional selective breeding of mouse populations for haloperidol-induced catalepsy. This was done in three mouse populations of increasingly complex genetic structure: an F2 intercross, a heterogeneous stock formed from four inbred strains (HS4), and a heterogeneous stock (HS-CC) formed by crossing the eight strains found in the collaborative cross. Brain (striatum) gene and genotype data were obtained using the Illumina platform. The genotype 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 biological mechanisms underlie differences in response to haloperidol. 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 MH 51372 and VA Research.

84C

Use of Pre-Collaborative Cross Mice to Characterize the Genomics of Allergen Response. Samir N. P. Kelada[1], Danielle Carpenter[1], David Aylor[2], Urraca Tavarez[1], Kari Kubalanza[1], Bailey Peck[1], John Didion[2], Darla Miller[2], Elissa Chesler[3], Gary Churchill[3], Fernando Pardo Manuel de Villena[2], David Schwartz[4], Francis Collins[1]. 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 extreme 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 Socs2, RAIDD/CRADD, and Plexin C1, is in a C57BL/6J background (C57BL/6J-*hghg*), however, complete absence of the ability to reproduce occurs when the homozygous *hg* locus is introgressed onto female FVB/NJ mice (FVB/NJ-*hghg*). This has 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 with genes in the hg locus and with reproduction. We utilize a novel biotechnique - recombinant backcross (BC) with selection to simultaneously uncover putative QTL regions and to fine-map the causative region(s) behind FVB/NJ-hghg female infertility. This method requires the mating of fertile female C57BL/6J-*hghg* mice and fertile FVB/NJ-*hghg* males. Their resulting female offspring are recurrently backcrossed to FVB/NJ-*hghg* males to introgress the FVB/NJ background onto the C57BL/6J background. Preservation of C57BL/6J loci that render females fertile are maintained by selecting female progenitors based on their ability to contribute offspring in the next generation. BC-5 mice will contain <2% C57BL/6J rescue loci. The backcross females will be genotyped using a high density SNP panel than can distinguish the FVB/NJ and C57BL/6J strains. We have produced BC-4 generation females. This genomic analysis is complemented with phenotyping data derived from FVB/NJ-*hghg* mice and FVB/NJ controls. Experiments that

investigated the: histology and architecture of the reproductive tract, hormonal profiles, functional reproduction traits, and ovarian gene expression indicate anovulation downstream of the FSH and LH pituitary pathways.

86B

Identification of Transcriptional Regulators at the *Meg3* Differentially Methylated Region. Erin N. McMurray, Jennifer V. Schmidt. Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL.

Genomic imprinting is the differential expression of two alleles of a gene based on their parent of origin. Many imprinted genes play a role in early development, and loss of imprinting can result in several types of disease and cancer. The *Dlk1* (*Delta-like 1*) - *Meg3* (*Maternally expressed gene 3*) locus is located on distal mouse chromosome 12. Proper expression of the genes at this locus is required for normal embryonic development, but the exact mechanisms regulating gene expression at this locus remain elusive. The *Meg3* differentially methylated region (DMR) begins approximately 1.5 kb upstream of the *Meg3* transcriptional start site and ends approximately 1.2 kb downstream in the first intron of the *Meg3* gene, and previous studies have shown that this DMR is required for proper imprinting of genes at the *Dlk1-Meg3* locus. The goal of this study was to determine the allele-specific chromatin profile of the *Meg3* DMR to provide a more complete picture of imprinting regulation at this locus. Chromatin immunoprecipitation (ChIP) experiments were conducted to identify histone modifications and/or transcription factors present at the *Meg3* DMR that may play a role in the regulation of gene expression at the *Dlk1-Meg3* domain. ChIP was carried out using embryonic day 12.5 (e12.5) embryos as well as ES cells. It was demonstrated that a number of histone modifications were present 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 *Meg3* expression. In ES cells, all modifications detected were distributed biallelically, corresponding to the bialleleic expression of the *Meg3* gene in ES cells. Transcription factors that have been shown to play roles at other imprinted loci were also detected 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 *Dlk1-Meg3* locus.

87C

The impact of transposable element variants on mouse genomes and traits. Christoffer Nellaker[1], Thomas Keane[2], Binnaz Yalcin[3], Kim Wong[2], Jonathan Flint[3], David Adams[2], Wayne Frankel[4], Chris P. Ponting[1]. 1) MRC Functional Genetics Unit, DPAG, University of Oxford, Oxford, UK; 2) Experimental Cancer Genetics, Welcome Trust Sanger Institute, Cambridge, UK; 3) Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK; 4) The Jackson Laboratory, Bar Harbor, USA.

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 time since the last common ancestor of Mus spretus and Mus musculus. Different families of TEVs exhibit, as expected, GC -dependent insertion biases. The more recent MuLV family, however, shows a bias for GC-rich sequence that sets it apart from other ERVs. We did not observe unambiguous evidence of purifying selection for any TEV family in these mice, except for within (1) genic exons, (2) promoters, and (3) certain housekeeping gene loci (for ERVs and LINEs). Enrichments of ERV and SINE densities upstream of genes are likely to reflect transcription-dependent insertional biases. Depletions of intronic TEVs in the sense direction could reflect strong selection or else a transcription-associated repair bias. Finally, we identify candidate TEVs for mouse traits, taking advantage of mouse brain transcriptome data and quantitative trait loci from heterogeneous stock mice. Our data and findings provide a firm foundation that will allow functional TEVs in the laboratory mice to be better understood.

88A

GWAS in mice. Jerome Nicod[1], Paul Potter[3], Carl Hassett[2], Xiangchao Gan[1], Barbara Nell[2], Russell Joynson[2], Hayley Page[2], Tertius Hough[2], James Cleak[1], Stuart Davidson[1], Polinka Hernandez-Pliego[1], Richard Mott[1], Tom Weaver[2], Jonathan Flint[1]. 1) Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom; 2) Mary Lyon Centre, MRC Harwell, Harwell Science and Innovation Campus, United Kingdom; 3) Mammalian Genetics Unit, MRC Harwell, Harwell Science and Innovation Campus, United Kingdom.

Genome-wide association studies (GWAS) have been extensively used for mapping complex traits in humans but have so far proved difficult to implement in mice because populations with the requisite characteristics are lacking. We have recently shown that some of the large colonies of outbred mice maintained by commercial breeders may be suitable for GWAS. A combination of relatively low linkage disequilibrium (LD) and lack of rare alleles makes commercially available outbreds an attractive resource for mouse GWAS (Yalcin et al, PLoS Genet, 2010). In addition, we have found that almost all of the genetic variants present in these populations can be found in classical inbreds, which mean that the genome of each outbred mouse can be reconstructed as a mosaic of inbreds 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 set of recombinant inbred strains and a small mouse diversity panel (MDP). PDNN corrected gene expression 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 loci. Supported by the FSU College of Medicine.

90C

Male reproductive phenotypes in extinct lines of the Collaborative Cross. F. Odet[1], D. L. Aylor[2], T. A. Bell[2], M. E. Calaway[2], C. Noneman[3], L. McMillan[3], F. Pardo-Manuel de Villena[2], D. A. O'Brien[1]. 1) Cell and Developmental Biology, University of North Carolina School of Medicine, Chapel Hill, NC; 2) Genetics, UNC, Chapel Hill, NC; 3) Computer Science, UNC, Chapel Hill, NC.

To assess potential causes of male infertility in extinct lines in the Collaborative Cross (CC), we have conducted reproductive phenotyping on >750 males from >390 funnels. These analyses include fertility status, testis weights and histology, seminal vesicle weights, epididymis weights, sperm counts, sperm and acrosomal morphology, and sperm motility as assessed by computer-assisted sperm analysis. We observed at least three classes of male infertility: (1) males with low testis weight, incomplete spermatogenesis and low sperm count, (2) males with normal testis weight, but defects in sperm motility and/or morphology and (3) males that cannot fertilize or support development, despite normal testis weight and normal sperm motility and morphology. Most traits exhibit a very wide range of variation. Some are normally distributed, including testis weights (8-660 mg) and mean curvilinear velocity of motile sperm (61-359 μm/sec), while others such as sperm counts (0-45 x 10^6/cauda epididymis) are not. Testis histology is also extremely variable in infertile males, ranging from normal spermatogenesis to partial or complete loss of germ cells. Additional phenotyping of the founder strains indicates that some of the testicular defects observed in the extinct lines, such as focal germ cell loss and vacuolization, are present in one or more of the founders. The high incidence of male infertility and the wide range of phenotypic defects observed, combined with the genetic structure of extinct and surviving CC lines, offer a unique opportunity to identify natural variation associated with male infertility. Supported by NIH R01 HD065024.

91A

Genetic Analysis of Genome-Scale Recombination Rate Evolution in Mice. Bret A. Payseur[1], Beth L. Dumont[1,2]. 1) Laboratory of Genetics, University of Wisconsin, Madison, WI; 2) Department of Genome Sciences, University of Washington, Seattle, WA.

Meiotic recombination ensures the proper segregation of homologous chromosomes and shapes the evolution of natural populations. Despite these functional roles, the rate of

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 Peck[1], Samir Kelada[1], David Aylor[2], Urraca Tavarez[1], Ryan Buus[2], Darla Miller[2,3], Elissa Chesler[3,4], Gary Churchill[4], Fernando Pardo Manuel de Villena[2], Francis Collins[1]. 1) National Human Genome Research Institute, NIH, 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 β-globin locus, which Peters et al. (2010) recently reported as causally linked to mean red cell hemoglobin concentration. We identified potential candidate genes for the remaining QTLs based on regional shared ancestry, gene ontology, and previously published data on genes within the confidence interval. Our results extend prior knowledge of the genetic basis of variation in blood cell numbers and size in mice.

93C

Chromosome-wide analysis of regional and local recombination rates in six mouse crosses. Petko M. Petkov[1], Evelyn Sargent[1], Timothy Billings[1], Terry Hassold[2], Karl Broman[3], Ken Paigen[1]. 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 which is 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 Prdm9, and studied the distribution of recombination events in female and male meiosis. Genetic lengths of these 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 functional genomics data for prioritization of candidate genes using the Ontological Discovery Environment. Vivek Philip[1,2], Jeremy Jay[1], Michael Langston[3], Erich Baker[4], Elissa Chesler[1,2]. 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 4,500 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 genomic 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 integrated with previously published QTL, GWAS and microarrays obtained from the following species: *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Drosophila melanogaster*, *Danio rerio* and *Macaca mulatta*. Using tools available within ODE, such as Jaccard Similarity, ABBA, 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 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. Powell[1], David L. Aylor[2], Wendy Foulds Mathes[2], Jill Steigerwalt[1], Daniel Pomp[2], Gary A. Churchill[2], Fernando Pardo-Manuel de Villena[2], David W. Threadgill[1]. 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) combined with that from founder strains (10-239 mg/kg/hr) 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 with 64 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 Praktiknjo, Bastien Llamas, MariePier 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.Y^A strain (whose Chr Y originates from the A/J mouse strain), we ourselves had reported that Chr Y polymorphisms modulate the biologic effects of post-pubertal testosterone in hearts. In the current study, we found that exposure to prenatal androgens was stronger in C57BL/6J than in C57.Y^A 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.YA. 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.Y^A 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.Y^A hearts with anti-AR antibodies. Out of 90 loci showing significant enrichment for AR, only 15/90 were common in both strains, whereas 52/90 and 23/90 showed enrichment only in C57BL/6J and C57.Y^A, 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 the effect of population structure on murine studies of pain sensitivity. Jill M. Recla[1,2], Vivek M. Philip[1], Raymond F. Robledo[1], Carol J. Bult[1], Elissa J. Chesler[1]. 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 RI, CC, and DO mice were phenotyped 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. Studying pain sensitivity in CC mice may allow for the identification of rare, relevant alleles that have become fixed through inbreeding, thereby boosting their frequency. 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

AComparison 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 must contain its own factor/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. Previous studies using quantitative trait loci (QTL) mapping identified three loci controlling susceptibility to seizure-induced cell death in mice derived from a C57BL/6J (B6;resistant strain) X FVB/NJ (FVB;susceptible strain) cross (Schauwecker et al., 2004). We previously constructed a congenic mouse, FVB.B6-*Sicd2* 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 subcongenics with smaller B6 donor regions that encompass *Sicd2* 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 *Sicd2* 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-*Sicd2d*. To help identify candidate genes, expression profiles of hippocampi from FVB (susceptible) and FVB.B6-*Sicd2d* (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-*Sicd2d* were located within the boundaries of the FVB.B6-*Sicd2d* 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 Sen[1], Hao Xiong[1], Evan Goulding[2], Elaine Carlson[1], Laurence Tecott[1], Charles McCulloch[1]. 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 observations. 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 flexible due to 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.

101B

Production and Use of Mice for the Center for Integrated Systems Genomics (CISGen). Ginger D. Shaw[1], Jason S. Spence[1], Timothy A. Bell[1], Alexander Vu[2], Sandra Hall[2], Leonard McMillan[2], Darla R. Miller[1], Fernando Pardo-Manuel de Villena[1]. 1) 1Lineberger Comprehensive Cancer Center, Dept. of Genetics, UNC at Chapel Hill, Chapel Hill, NC; 2) 2Dept. 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, huts, balls, and chew toys in a cage). 495 mice have been tested for sociability and anxiety from 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 (Baric, Heise), craniofacial morphology (Facebase Consortium), cancer and drug toxicity susceptibility (Threadgill).

102C

High Resolution Genomic Architecture of Genetic Reference Populations: Chromosome Substitution Panels and Recombinant Inbred Strains. Petr Simecek[1], Jiri Forejt[1], Robert W. Williams[2], Lu Lu[2], Thomas E. Johnson[3], Beth Bennett[3], Brad Rikke[3], Christian F. Deschepper[4], Marie-Pier Scott-Boyer[4], Gary Churchill[5], Fernando Pardo-Manuel de Villena[6]. 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-Chr#PWD/Ph/ForeJ and C57BL/6J-Chr#A/J/NaJ) 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 DNA sample. Given that MDA can achieve a map 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 line 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 arise 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 mapping in 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 animal with an average spacing of 10.2 megabases. Animals were phenotyped 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. Tarantino[1], Robin B. Ervin[1], Hongzhe Duan[1], Steve Cook[2], William C. Zamboni[2], Wonil Chung[3], Fei Zou[3], Tim Wiltshire[2]. 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 the extent of genetic and environmental influences on complex behavioral phenotypes. Recent technological advances and the resulting genomic information now available in the mouse have expanded the usefulness of inbred strain phenotypic data. Dense SNP haplotype maps can now be used in conjunction with phenotypic data to map genomic loci involved in complex phenotypes and also to replicate and narrow previously identified loci. Our laboratory recently completed a survey of initial locomotor sensitivity to cocaine in 45 inbred strains. The data from these studies show extensive phenotypic diversity. Most behaviors exhibit moderate to high heritability, making them amenable to genetic analysis. Using an algorithm for associating phenotypes with haplotype status, we have identified loci that are linked to the behavioral differences observed among inbred strains. We are also collecting gene expression data from all 45 strains from brain regions in drug reward pathways to identify expression QTL that overlap with mapped behavioral loci. Finally, we have examined cocaine pharmacokinetics at two time points in the same set of inbred strains to determine the extent to which PK differences explain behavioral output. Our data indicate that pharmacokinetics have some influence on behavioral response to cocaine - particularly in strains at the extremes of the phenotypic distribution. However, PK differences alone do not explain strain variability in locomotor response to cocaine.

105C

Comparison of variant detections using whole genome sequencing of the DBA/2J mouse strain. Xusheng Wang[1], Megan Mulligan[1], Khyobeni Mozhui[1], Lu Lu[1], Zugen Chen[2], Stanley Nelson[2], Williams Taylor[1], Robert Williams[1]. 1) Anatomy and Neurobiology, UTHSC, Memphis, TN., TN 38163; 2) Department of Human Genetics, University of California, Los Angeles, CA 90095.

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 (~99.7% 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 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 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.

106A

A combinatorial RNAi screen of BAF-associated factors reveals genetic interactions regulating self-renewal. Samuel Wormald, Peri Tate, Stefania Borchia, Daniel Turner, William Skarnes. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

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.

107B

Parent of Origin Effects in the Mouse. Wei Yuan, Andrew Edwards, Xiangchao Gan, Binnaz Yalcin, Guo-Jen Huang, James Cleak, Jonathan Flint, Richard Mott. Wellcome Trust Centre for Human Genetics, Oxford University , Oxford, United Kingdom.

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 (*Man1a2*), 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.

108C

Mice lacking *Cappuccino*, a Hermansky-Pudlak syndrome associated protein, exhibit a decrease in bone mass. Cheryl L. Ackert-Bicknell[1], Jordanne Dunn[1], Luanne L. Peters[2]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY.

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/+* and *cno/cno* mice. In short, calvarial osteoblasts were isolated from neonatal *cno/cno* and *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 have an increased mineralizing ability whereas in vivo, mice lacking CNO protein exhibit an osteopenic phenotype, and exhibit an increase in osteoclast number. Thus, CNO may have a role in coupling bone formation with bone resorption.

109A

A mouse model for juvenile hydrocephalus. Oliver Appelbe, Jenniffer Ramalie, Ekaterina Steshina, Ali Attarwala, Lindy Triebes, Jennifer Schmidt. University of Illinois at Chicago, Chicago, IL.

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 contains 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.

110B

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 115 mutant lines that had a wide range of phenotypes. Of these, 75 caused embryonic or postnatal lethality. Mutant characterization revealed defects in gastrulation, placental formation, palate formation, nervous system development, hematopoiesis and cardiovascular development. Our lab is particularly interested in defects in hematopoiesis and cardiovascular development. The *l11Jus13* lethal line was of particular interest to our lab. Homozygous mice had craniofacial, heart, and blood defects that resulted in death after birth, and heterozygous mice developed leukemia or autoinflammation. Subsequent studies revealed that a hypomorphic mutation in nucleoredoxin (*Nxn*), a disulfide oxidoreductase, was responsible for the wide range of birth defects. We examined the hematopoietic system to test the impact of this mutation on hematopoietic stem cell function (HSC). *NxnJ13/J13* mice had many blood defects in lymphoid and myeloid lineages. However, mice that live long enough to analyze blood parameters were rare in this allele. When HSC cells 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-βgeo-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.

111C

Identification of Quantitative Trait Loci Controlling Tumor Morphology in a Mouse Model of Human Colorectal Cancer. David J. Bautz, David W. Threadgill. Department of Genetics, 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 polypoid 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. Using the azoxymethane (AOM) model of human CRC we find that I/LNJ mice develop approximately 85% flat tumors while KK/HlJ mice develop approximately 85% polypoid tumors. Importantly, microarray analysis of flat and polypoid tumors from both strains indicates that genomic differences influencing tumor morphology are host specific and not determined by the developing tumors. F1 hybrids between the strains developed 50% flat lesions. To identify genetic loci contributing to these phenotypic differences, we established an F2 cross between these two mouse strains consisting of 340 animals and in the first 64 mice found they develop approximately 40% flat tumors. These mice have been genotyped at 110 SNP's evenly spaced across the genome and statistical analysis has identified two suggestive QTL for tumor morphology, one on Chromosome 9 and the other on Chromosome 12. Additional data will be presented on the larger F2 cohort. eQTL analysis of normal colon tissue is also being performed to fine map and identify candidate genes at these QTL. Our preliminary results indicate that tumor morphology is controlled by host genetic factors and we anticipate future studies focusing on the identification of the specific factors for development of therapeutic interventions.

112A

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 30-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 32mb 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.

113B

Transgenic- and Obesity-Induced Overexpression of miRNA-143 leads to Insulin Resistance. Vincent Beuger[1], Jost Seibler[1], Sabine Jordan[2], Jens Brüning[2], Diana Willmes[2], Nora Redemann[2], Thomas Wunderlich[2], Hella Brönneke[3], Carsten Merkwirth[4], Hamid Kashkar[5], Vesa Olkkonen[6], Markus Krüger[7], Thomas Böttger[7], Thomas Braun[7]. 1) TaconicArtemis GmbH, Neurather Ring 1, D-51063 Cologne, Germany; 2) Department of Mouse Genetics and Metabolism, Institute for Genetics, Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD) and Center of Molecular Medicine Cologne (CMMC) University of Cologne, and 2nd Department for; 3)

Phenotyping facility of the Cologne Excellence Cluster on Cellular Stress Responses in Aging; 4) Institute for Genetics, Cologne Excellence Cluster on Cellular Stress Responses in Aging Associated Diseases (CECAD) and Center of Molecular Medicine Cologne (CMMC), D-50674 Cologne, Germany; 5) Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne; 6) Minerva Foundation Institute for Medical Research, and National Institute for Health and Welfare; 7) Max-Planck-Institute for Heart and Lung Research, D-61231 Bad Nauheim, Germany.
The contribution of dysregulated microRNA expression to the development of type 2 diabetes mellitus so far remains elusive. We have observed that the transcription of miR-143 and -145 is upregulated in liver of dietary mouse models of obesity. Induced transgenic overexpression of miR-143, but not of miR-145 causes insulin resistance and impaired insulin stimulated AKT activation. Conversely, mice deficient for the miR-143/145-cluster are protected from the development of obesity-associated insulin resistance. Quantitative mass spectrometry based analysis of protein expression in liver of miR-143 transgenic and control mice revealed miR-143-dependent downregulation of oxysterol-binding protein-related protein (ORP) 8. Our approach for the inducible expression of small RNA molecules in mice will enable new insights into miRNA function and molecular disease mechanisms.

114C

Generation of CreERT2 transgenic mouse lines for time and cell specific conditional gene inactivation. Marie-Christine Birling, Lydie Venteo, Olivia Wendling, Yann Hérault, Guillaume Pavlovic. Genetic Engineering, Institut Clinique de la Souris, 1, rue Laurent Frie, 67400 Illkirch, France.
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 and time controlled 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/altered only in this 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 deleter 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/.

115A

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 mouse models of 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.

116B

Molecular mechanisms altering skeletal development and homeostasis in Ts65Dn Down Syndrome Mice. Joshua D. Blazek, Samantha L. Deitz, Randall J. Roper. Department of Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN.
Down syndrome (DS) is caused by three copies of human chromosome 21 (HSA21) and results in abnormal craniofacial and appendicular bone phenotypes. The Ts65Dn mouse model contains three copies of nearly half of the genes found on HSA21, and exhibits craniofacial skeletal phenotypes similar to those observed in humans with DS. We recently demonstrated abnormalities in the development and homeostasis of the appendicular skeleton of Ts65Dn mice. Femurs from trisomic mice exhibit alterations in trabecular bone architecture and overall bone strength. Furthermore, bone formation rates were found to be significantly reduced, suggesting trisomy impacts bone development and maintenance in Ts65Dn mice and by extension humans with DS. *DYRK1A* is triplicated in both humans with DS and Ts65Dn mice and its protein acts as a kinase critical during development. DYRK1A negatively regulates the nuclear localization and activation of NFATC, a transcription factor critical to signaling pathways associated with cell proliferation and bone development, and is overexpressed in the E9.5 Ts65Dn mandible precursor. We hypothesize that the disruption of NFATC signaling contributes to the abnormal bone phenotypes observed in Ts65Dn mice. To test our hypothesis, we are quantifying NFATC cellular localization, cellular differentiation potential in the mandibular precursor, as well as NFAT localization and cellular differentiation in the E17.5 cartilage templates. Our preliminary results show a higher ratio of cytoplasmic localization of NFATC in trisomic compared to euploid E9.5 embryos. Understanding the molecular mechanisms underlying DS bone phenotypes may help improve the quality of life for individuals with DS.

117C

Haploinsufficient Role for Pax2/Emx2 in Vesicoureteral Reflux and Other CAKUT-like Malformations. Sami K. Boualia[1], Yaned Gaitan[1], Inga Murawski[2], Robert Nadon[3], Indra R. Gupta[2], Maxime Bouchard[1]. 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, bifid ureter and a high penetrance of VUR. Remarkably, most compound heterozygous mice display low intravesical pressure reflux. Early analysis of *Pax2+/-;Emx2+/-* embryos identify ureter budding defects as the primary cause of urinary tract anomalies. 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 VUR and CAKUT in humans.

118A

A 1.7Mb interval on mouse Chromosome 3 confers susceptibility to *Helicobacter hepaticus*-induced innate colitis and associated colon cancer. Olivier BOULARD, Stefanie KIRCHBERGER, Daniel J. ROYSTON, Fiona M. POWRIE. TGU, NDM, University of Oxford, Oxford, United Kingdom.
Chronic inflammation of the intestine (observed in Crohn's disease and ulcerative colitis) has been associated with an increased risk to develop colorectal cancer. Although the genetics of inflammatory bowel disease (IBD) has greatly progressed over recent years, precise identification has yet to be achieved for most of the susceptibility genes. Furthermore, little is known about the genetic factors that influence the progression to colorectal cancer in IBD patients. Animal models could provide valuable insight in this field. As such, innate typhlocolitis and associated colon cancer can be induced in the susceptible strain 129S6.RAG$^{-/-}$ but not in the resistant C57BL/6.RAG$^{-/-}$ strain, upon chronic infection with the single pathogen *Helicobacter hepaticus*. By generating a novel congenic line, we identified a major susceptibility locus for *H. hepaticus*-induced colitis in a telomeric region of Chromosome 3. Fine mapping of the 1.7 Mb susceptibility interval was achieved by development of recombinant lines and SNPs genotyping. Systematic sequencing and expression analysis of the candidate genes (and microRNAs) in the interval have not identified a definitive element, but pointed toward the gene *Alpk1* (Alpha kinase 1) as the best candidate so far. Furthermore, reciprocal bone-marrow chimera experiments revealed that the susceptibility locus was acting exclusively in the hematopoietic compartment. Finally, the congenic interval was found to decrease the frequency of colitis-associated neoplasia, using a tumor-promoting model combining chronic *H. hepaticus* infection and the chemical carcinogen azoxymethane (AOM). Although the syntenic region on human chromosome 4q25 has not been so far implicated in IBD or colorectal cancer predisposition, our study may open new pathways and therapeutic perspectives for chronic inflammatory disorders and associated cancer.

119B

Improved Resolution across the *Bbaa2* Murine Lyme Arthritis Susceptibility Locus by SNP Based Genotyping. Kenneth K. C. Bramwell[1], Ying Ma[1], John H. Weis[1], Cory Teuscher[2], Janis J. Weis[1]. 1) University of Utah; 2) University of Vermont.

Abstract: Lyme arthritis is caused by infection with 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 metabolic syndrome in humans. Gudrun A. Brockmann, Claudia Hantschel, Christina Neuschl, 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. Buchovecky, 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 *Sqle*, *Lss* and *Sc4mol* as well as a number of other genes involved in cholesterol biosynthesis. Members of the steroid 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 Huntingtons. 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. Bunton-Stasyshyn[1], Anny Devoy[1], Victor L. J. Tybulewicz[2], Elizabeth M. C. Fisher[1]. 1) Department of Neurodegenerative Disease, Institute of Neurology, UCL, London, United Kingdom; 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 the enzyme Cu/Zn superoxide dismutase (SOD1), the largest proportion of ALS accounted for by a single gene. There are currently 12 disease associated SOD1 mutations in published transgenic mouse models. These have been useful tools 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 pathology and 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.

123C

A genetic approach to α-synuclein function. Deborah E. Cabin, MegAnne 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 *Atp7a*, the X-linked gene mutated in Menkes disease. *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-*Prdm14* knock-in mouse model and intraductal injection of a self-deleting lentiviral Cre recombinase for the modeling of

tumor initiation in breast cancer. Brandi L. Carofino[1,2], Monica J. Justice[2]. 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 mammary-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-EGFP marker. When knocked-in to the ROSA26 locus, this construct allows for spatial and temporal Cre-mediated excision of the STOP cassette and activation of oncogene expression. 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 mammary-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 tumor clonogenicity and transplantability.

125B

The influence of α-synuclein on Aβ 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 pure 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 plaque. 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 quantify and compare 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 transgene for a doubly mutant form of APP 695 (TgCRND8) onto our *Snca* null mice; use of this transgene allows us to begin plaque analysis 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 plaque load in the absence of Snca is not replicated using TgCRND8 on the 129S6 genetic background. Complete analysis will allow us to compare the influences of mouse and human SNCAs 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. Chen[1], B. Willis[1], A. Cipollone[1], E. Engelhard[1], K. Drake[2], K. Lloyd[1], D. West[1,3]. 1) Mouse Biology Program, University of California, Davis, CA; 2) Seralogix 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 literature, and in two Riken clone KOMP mutants of unknown function: *1300002K09Rik* and *1700029I15Rik*. For each mutant, we used at least 3 ~ 50-day-old males 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 Seralogix using a Bayesian approach (www.seralogix.com/). For *db/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 phenoyptes 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. *Cyp4a14* was up regulated more than 6-fold and hit 4 pathways (retinol metabolism, PPAR signaling, arachidonic acid metabolism and fatty acid metabolism) with 3 other genes also affected in these pathways. For *1700029I15Rik* KO mice, 212 genes were up- and 396 were down-regulated in 4 different tissues. The most effected pathways in *1700029I15Rik* 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 utility of compensatory transcriptome analyses for predicting gene function. Supported by NIH Grants: 3U01HG004080-04S1, 3U42RR024244-03S2.

127A

QTL Mapping in Advanced Intercross Lines: Statistical and Computational Issues. Riyan Cheng, Abraham Palmer. Department of Human Genetics, The University of Chicago, Chicago, IL.

Advanced intercross lines (AILs) are an ideal resource for fine-scale quantitative trait locus (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 over 34 generations. We studied methods including permutation and gene dropping for empirical significance thresholds using extensive simulations under different statistical models, and found that these methods worked well when the model appropriately incorporated relatedness information but would fail to control type I error rates if relatedness was ignored. A genome-scan can involve manipulation of large matrices, depending on the statistical model, and thus be computationally demanding. We contributed an R package "QTLRel" to R-CRAN that is capable of fast genome-scans and related analysis of data similar to ours. The issues addressed here can extend to data from nuclear families or a pedigree where relatedness is a concern.

128B

Genetic dissection of resistance to *Yersinia pestis* of SEG/Pas mice. Lucie Chevallier[1], Charlène Blanchet[3], Jean Jaubert[1], Emilia Pachulec[2], Christian Demeure[2], Claudia Pommerenke[4], Jean-Marc Cavaillon[3], Klaus Schughart[4], Elisabeth Carniel[2], Jean-Jacques Panthier[1], Xavier Montagutelli[1]. 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 infection 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 therapies. We showed that SEG/Pas mice, derived from *Mus spretus*, are resistant to a subcutaneous inoculation of 100 *Y. pestis* bacteria, whereas all classical laboratory strains tested are susceptible. A backcross between SEG/Pas and C57BL/6J 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 congenic strains carrying SEG alleles at each of the three QTLs in a C57BL/6 background. Bi- and tri-congenic strains were also created by crossing congenics. Survival and time to death were characterized for each of these strains. Our results indicate that the males and females triple congenics survive to about 32% while all C57BL/6 die. Since this is much less than the survival rate observed in the backcross mice 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 congenic strains will enhance our ability to unravel the mechanisms involved in resistance to plague in SEG/Pas.

129C

Functional characterization of three loci (*Ses*1, *Ses*4 and *Ses*5). Marie Chevenon[1,3], Mayss Naccache[4], Megan Eva[2,3], Danielle Malo[1,2,3]. 1) Experimental Medicine; 2) Human Genetics; 3) Complex Traits Group, McGill University, Montreal, QC, Canada; 4) P3G, Montreal, QC, Canada.

Salmonella Typhimurium and *S.* Enteritidis cause a food-borne disease resulting in gastroenteritis. To study the persistence of *Salmonella* during the late phase of infection, a mouse model was developed using C57BL/6J (B6) mice that clear the bacteria completely from the spleen and lymph nodes within 42 days post-infection and 129S6/SvEvTac (129S6) mice that become chronic carriers. Linkage analyses using a cross between B6 and 129S6 mice led to the mapping of ten quantitative trait loci, *Ses*1 (*Salmonella*

Enteritidis susceptibility locus 1) to *Ses*10, associated with high bacterial load in 129S6 mice. In the females, both *Ses*1 and *Ses*3 showed significant effects on bacterial clearance and two significant interactions between *Ses*1 and *Ses*4 and between *Ses*1 and *Ses*5 accounted for 72% of the total phenotypic variation. We have created congenic mouse strains by introgressing specific B6 chromosomal regions onto 129S6 background for *Ses*1, *Ses*3, *Ses*4 and *Ses*5. Phenotypic analysis confirmed that *Ses*1 contributes to bacterial clearance in both sexes. *Slc11a1* was shown to be the gene underlying *Ses*1 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_H2 response compared to a strong T_H1 response in 129S6 mice. To functionally validate in vivo the interaction between *Ses*1 and *Ses*4 or *Ses*5, we created double congenic mice. Bacterial counts in the spleen and the liver demonstrated that both the 129S6.B6-*Ses*1/*Ses*4 and the 129S6.B6-*Ses*1/*Ses*5 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. Cheverud[1], Jane Kenney-Hunt[1], Hatti Hyler[1], Connie Shao[1], Lorri Leung[1], Linda Sandell[2], Matthew Silva[2]. 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 3 of which were also significant for fat. Positional candidate genes and sequence and expression variations are considered to aid in defining the quantitative trait gene.

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. The former causes degeneration of photoreceptor neurons, whereas the latter leads to loss of dopaminergic neurons (DA). This study aims at defining the role of insufficiency of RAN-binding protein -2 (RANBP2) in promoting neuroprotection in response to chronic light and exposure to MPTP. Wild-type and inbred haploinsufficient *Ranbp2* mice were exposed to cyclic/low light conditions, chronic light or acute MPTP. Immunohistochemistry of the retina and brain was used to assess changes in cell populations and their molecular state. A metabolomics was also used to identify signature markers between wild-type and *Ranbp2*$^{+/-}$ mice exposed to MPTP. Haploinsufficiency of *Ranp2* protects photoreceptors and the retinal pigment epithelium (RPE) from degeneration by light damage, whereas it increases and decreases the susceptibility of DA neurons of the brain and retina to MPTP toxicity, respectively. *Ranbp2*$^{+/-}$ mice upon light-elicited oxidative stress suppresses membrane dysgenesis and modulates the levels of a subset of partners of Ranbp2, ubiquitylated proteins and a set of orphan nuclear receptors. *Ranbp2*$^{+/-}$ suppresses also the oxidative stress-induced formation of lipid deposits in the RPE. On the other hand, MPTP in *Ranbp2*$^{+/-}$ mice decreases the locomotor activity and leads to transient damage of TH^+-neurons of the SNpc, whereas it promotes an increase of $GFAP^+$-glial cells and TH^+-amacrine neurons of the retina. Ten brain metabolites were also significantly modulated by MPTP between wild-type and *Ranbp2*$^{+/-}$ mice. Hence, haploinsufficiency of *Ranbp2* promotes the cell-type-dependent neuroprotection and regulation of proteostasis and lipid homeostasis acting on functionally interconnected pathways in response to oxidative stress.

132C

INVESTIGATION OF MYOTILIN AS A MODIFIER GENE IN A MOUSE MODEL OF CARDIOMYOPATHY. Pei-Lun Chu[1,2], Lan Mao[3], Howard Rockman[3], Olli Carpen[4], Douglas Marchuk[1,2]. 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 Medicine, Duke University, Durham, NC, USA; 4) 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-interpreting sensor 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 loss of 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 segmentation clock function 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 requires the periodic expression of genes such as Lunatic fringe (*Lfng*), which encodes a glycosyltransferase that modulates Notch signaling. In order for the LFNG protein to function in 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 the functional relevance of this processing we created a novel targeted allele of *Lfng* that tethers the protein in the Golgi, preventing protein processing 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-translation mechanism allowing tight temporal and spatial modulation of Notch signaling during somitogenesis. Further, this novel hyperactive *Lfng* allele can be used to examine the functions of LFNG during other developmental decisions.

134B

c-Fos Induction Associated With Ethanol Withdrawal In Chromosome 1 Congenic and GIRK3 Knockout Mice. Alexandre M. Colville[1], Gang Chen[1], Laura Kozell[1,2], Kari Buck[1,2]. 1) Behavioral Neuroscience, OHSU, Portland, OR; 2) Portland Alcohol Research Center, VA Medical Center, Portland, OR.

Our goal is to dissect the neural and molecular substrates by which quantitative trait loci (QTLs) influence ethanol dependence and associated withdrawal. Using mapping populations derived from DBA/2J (severe EtOH withdrawal phenotype) and C57BL/6J (mild EtOH withdrawal phenotype) mice, we previously identified QTLs on distal Chromosome 1 with large effects on chronic and acute ethanol withdrawal. We created a congenic strain with this segment of Chr 1 from the 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 *Kcnj9* (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 29:11662, 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 ($p=\sim0.1$) 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 Chr 1 QTL effects 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 (A/J). 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 ($P < 10\text{-}5$) 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, and QTL for work on Chromosomes 1, 2, 4, 10, 11 and 16. Two significant associations on Chromosome 11 (~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 white spots (*Rwhs*) is an 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 has 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 *Myo1d* gene. Sequencing of the entire interval identified one nucleotide substitution which changes a highly-conserved isoleucine to threonine (I135T) in *Tmem98*, 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 recombineered to contain the I135T amino acid substitution can also rescue M1L lethality. All these results suggest that the lethal diaphragmatic hernia phenotype in *Rwhs* homozgyotes is not caused by a straightforward detrimental effect on *Tmem98* function caused by the isoleucine to threoine 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 *Nramp1*) gene to modulate pristane-induced arthritis in mice. Marcelo De Franco[1], Antonella Galvan[2], Luciana Peters[1], Tatiane Canhamero[1], Francisca Vorraro[1], Andrea Borrego[1], Jose Jensen[1], Wafa Cabrera[1], Nancy Starobinas[1], Orlando Ribeiro[1], Cristiano Rossato[1], Tommaso Dragani[2], Olga Ibañez[1]. 1) Laboratorio de Imunogenetica, Instituto Butantan, Sao Paulo, Sao Paulo, Brazil; 2) Department of Experimental Oncology, Istituto Nazionale Tumori, Milan, Italy.

AIRmax (maximum inflammation) and AIRmin (minimum inflammation) phenotype selected mice showed distinct susceptibility to pristane-induced arthritis (PIA). AIRmax^{SS} 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 bioarrays (27k genes) using individuals mRNA (n=4) of arthritic or control paws. In parallel, genome wide association studies were performed to evidence arthritis QTL in F2 (AIRmax x AIRmin) 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 AIRmax^{RR} and AIRmax^{SS} mice, as well as in AIRmax heterozygous group. Higher up-regulation of chemokines *Cxcl1, Cxcl9, Cxcl5, Cxcl13* genes on Chromosome 5 were observed in AIRmax^{SS} than in the other lines. In Chromosome 8, macrophage scavenger receptor 1 and heme oxigenase (decycling) 1 genes were also more expressed in AIRmax^{SS} 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 AIRmax^{SS} 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 responsible 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. Although their ejaculates are similar to wild type in terms of sperm count, morphology, and motility, and they are able to fertilize oocytes, no embryos sired by knockout males implant in the female's uterine horns. Failure to implant indicates that the copulatory plug contributes to some minimum 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 (MGP) 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 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/mouseportal). 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 Albino rats fed ethanol extract of *Phyllanthus amarus*. HANNAH EDIM ETTA[1], ENEOBONG EFFIOM ENEOBONG[1], PAUL BASSEY UDOH[2], BASSEY OKON[3]. 1) BIOLOGICAL SCIENCE, CROSS RIVER UNIVERSITY OF TECHNOLOGY, CALABAR, NIGERIA; 2) ENVIRONMENTAL BIOLOGY

AND ZOOLOGY DEPARTMENT, UNIVERSITY OF CALABAR, NIGERIA; 3) ANIMAL SCIENCE DEPARTMENT, UNIVERSITY OF CALABAR, NIGERIA.
The effect of a well known herb, *Phyllanthus amarus* on the sperm characteristics in albino rats was studied. This was an investigation of the age-long claim by the locales in the rural communities in the southern states of Nigeria where this plant is consumed religiously that it affects sperm quality, hence sexual potency in males. Ethanol extract of *P.amarus* in graded doses of 70mg/kg, 140mg/kg and 210mg/kg BW were administered by oral gavage to the experimental animals for 14 days. At the end of the administration, epididymal sperm was collected and analyzed. Sperm analyses involved sperm count, sperm morphology test and sperm motility test. The Neubuers' haemocytometer was used to analyse the sperm count; sperm motility analyses were done using a wet mount while the sperm morphology test was done by staining air-dried and fixed smears with haematoxylin/eosin stain before viewing under a high power microscope. At the doses administered, *P. amarus* extract affected the sperm number, morphology and motility of treated animals. Epididymal sperm count and motility were significantly reduced ($P<0.05$). Compared to the control mean sperm count of 6.39×10^6, groups II, III and IV rats had mean sperm counts of 4.56×10^6, 3.67×10^6 and 2.5×10^6 respectively. Sperm motility scores were 75.0%, 71.67%, 66.67% and 50.0% for group I (control), groups II ,III and IV respectively. Abnormal sperm morphologies like detached heads, fusion sperm, and mis-shappened heads with hook at wrong angles were observed. The present investigation shows that at high doses, ethanol extract of *P. amarus* lowers sperm quality in albino rats thus conferring antifertility properties on the rats. This proves that the claim that *P. amarus* affects sexual potency in man may be true. Further research in this regard is recommended.

141C
Host polymorphisms contribute to differential disease phenotypes in response to two respiratory viruses: Influenza A Virus and SARS Coronavirus. Martin T. Ferris[1,2], Lisa Gralinski[1,3], David L. Aylor[2], Ryan Buus[2], Alan C. Whitmore[1], Fernando Pardo-Manuel de Villena[2], Ralph S. Baric[1], Mark T. Heise[1,2,3]. 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. Confirming the unique patterns of population-wide disease variation, 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.

142A
Proteomic identification of protein misexpression during cardiogenesis in the Ts65Dn Down syndrome mouse model. Arianna Franca, Erik M. Kelly, Clara S. Moore. Biology Dept, Franklin and Marshall College, Lancaster, PA.
Down syndrome (DS), a complex genetic disorder in humans, is due to the triplication of human chromosome 21 (*Hsa*21) 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 ezrin, 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 respresents 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.

143B
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 Sanger Mouse Genetics Project (MGP) 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. 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 phenodeviants 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.

144C
ENU mutagenesis identifies mouse mutants with cortical patterning defects resembling human brain malformation syndromes. Seungshin Ha[1,2], Rolf W. Stottmann[1,2], Amy Bernard[3], David R. Beier[1,2]. 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 (*Rgs4-lacZ*) or in a subset of cortical axons (*TAG-1-tau-lacZ*). Second, we employed a high-throughput RNA *in situ* hybridization of layer-specific markers as a primary screening tool. To date, 54 lines have been 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 corticofugal 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 proved 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.

145A
Deciphering the complex genetic interactions contributing to learning and memory phenotypes in Down syndrome mouse models. Yann Herault[1,2,3], Arnaud Duchon[1], Damien Maréchal[1], Claire Chevalier[1], Patricia Lopes[1], Ignasi Abisanda[4], Victor Tybulewicz[5], Elizabeth Fisher[6], Mara Dierssen[4], Sabrina Luilier[7], Jean-Charles Bizot[7], Stylianos Antonarakis[8], Veronique Brault[1]. 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, TAAM, CNRS, UPS44, Orléans, France; 4) Genes and Disease Program, Center for Genomic

Regulation, CIBER de Enfermedades Raras, 08003 Barcelona, Spain; 5) MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, UK; 6) UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK; 7) Key-Obs S.A., Orleans, France; 8) Department of Genetic Medicine and Development, University of Geneva Medical School, 1 Rue Michel-Servet, 1211 Geneva, Switzerland.
The underlying genetic basic of the genotypes - phenotypes relationship in Down syndrome (DS) is still a challenge. Our current working hypothesis is based on interactions between "dosage sensitive" genes or regions spread along the HSA21 are responsible for the complex feature of the pathology. Orthologuous genes on HSA21 are located on mouse Chromosomes (MMU) 16, 17 and 10. In order to go further and to decipher the gene interactions involved in DS phenotypes, we generated several trisomies and monosomies in the mouse for the different regions homologous to HSA21. We used additive (combining duplications) and substractive strategies (deletion combined to larger trisomies) to decipher the contribution of 4 regions in the induction of DS phenotypes. We will report here the detailed phenotypic analysis of new models and the contribution of several regions to the various DS induced phenotypes, affecting the locomotor system, learning and memory and the morphology. In parallel to the functional analysis, we generate a series of expression analysis to identify the dosage sensitive genes and the pathways that are affected. The data generated challenge our current hypothesis, but also point to new therapeutic approaches.

146B
Mapping the Genetic Determinants of Microvasculature. Bruce Herron[1], Krista Morales[1], Leahana Rowhel[1], Barbara Beyer[1], Fang Liu[2], Jason Smith[1], Richard Cole[1]. 1) Molecular Toxicology, Wadsworth Center, Albany, NY; 2) Dept. of Dermatology, University of Pennsylvania, Philadelphia, PA.
The identification of novel genetic factors that mediate angiogenesis in mammals will provide a critical resource for new therapies and biomarkers that can either treat or predict the severity of many diseases in humans. However, angiogenesis is a complex trait that is mediated by multiple environmental and genetic factors. While mapping the genetic polymorphisms that contribute to complex traits to Quantitative Trait Loci (QTLs) is now commonplace, identification for the underlying mutations remains a challenge. Central to the challenge of QTL identification is that most assays used to detect differences in angiogenesis measure late endpoints that are dependent multiple QTLs while efficient positional cloning strategies are designed to detect Mendelian effects. Accordingly, efficient identification of angiogenesis QTLs would greatly benefit from designing phenotypic assays that measure early events in vessel development that are presumably controlled by fewer genetic factors. We have developed an Ex vivo assay that can measure subtle differences in microvasculature angiogenesis. This method has revealed genetic heterogeneity among inbred strains of mice that can be mapped to specific QTLs. We will present data from two crosses that each show a single large effect QTL, indicating that this approach should ultimately lead to a greater likelihood of success for positional cloning.

147C
Genome-wide mutation analysis in a mouse mimic of human aging-associated neurodegeneration using the novel Mouse Diversity Genotyping Array (MDGA). Kathleen A. Hill, Susan T. Eitutis, Andrea E. Wishart. Dept Biol,Biol & Geol Sci Bldg, Univ Western Ontario, London, ON, Canada.
Our research provided evidence that mutations occur in synchronous clusters or showers in mammalian genes [PNAS 104:8403-8]. We now hypothesize that mutation showers previously observed in single locus mutation targets are in fact numerous and pervasive across the genome. Fortunately, genome-wide mutation load can now be examined using the Mouse Diversity Genotyping Array (MDGA) for 623,124 known SNPs [Nat Meth 6:663-6]. In this first application of the array for mutation research, we determined the number of SNP changes between spleen and cerebellum of the same mouse and repeated this for four mice. Somatic mutations were detected as "on target" alterations in a SNP allele and "off target" mutations resulting in "no allele" calls between spleen and cerebellum. Spleen and cerebellum had comparable mutation loads in all mice. We also applied the MDGA to genotype DNA isolated from spleen and cerebellum for the harlequin (*hq*) mouse model of premature aging in comparison to age-matched wild type mice. Knockdown of the apoptosis-inducing factor (*Aif*) gene in *hq* mice results in mitochondrial dysfunction with elevated reactive oxygen species and dramatic cerebellar neuron loss. Mutation load was significantly greater in *hq* mice compared to age-matched wild type mice (Fisher's exact test; $p < 0.001$) and more frequent on chromosomes 12, 13, and 14 ($p < 0.001$) in both spleen and cerebellum. This novel application of the MDGA shows potential for characterizing genome instability, identifying local mutation hotspots and understanding causal links between genome instability and aging-associated disease.

148A
Identifying cis regulatory elements at the imprinted *H19/Igf2* locus. Folami Y. Ideraabdullah, Joanne Thorvaldsen, Sherry Wen, Marisa Bartolomei. Cell and Developmental Biology, University of Pennsylvania, Philadelphia, PA.
Genomic imprinting is an important form of epigenetic gene regulation that results in expression from a single allele in a parent of origin dependent manner. Loss of imprinting (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 endodermal 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 (ΔIVS) or 1.3kb (Δ2,3) 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 Δ2,3 mutant tissues of mesodermal origin revealing a novel relationship between ICR-mediated insulation and the downstream enhancers. Studies are ongoing to dissect the underlying mechanism of this spatially restricted disruption of insulation and imprinting.

149B
The Power of the Collaborative Cross mouse resource population for mapping QTL associated with host susceptibility to Klebsiella pneumonaie infection. Fuad Iraqi[1], Karin Vered[1], Binnaz Yalcin[2], Itzhak Ofek[1], Caroline Durrant[2], Richard Mott[2]. 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 pneumonaie (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 104 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. Survival analysis has shown that different CC lines differed significantly ($P<0.05$) with spectrum of mean survival time between 1 to 12 days post infection. "Broad sense" heritability (including epistatic, but not dominance effects) of this trait was high as of 0.45. Permutation test analysis determined the 50%, 90% and 95% threshold levels and found to be 6.2, 8.1 and 8.8, respectively. Two significant QTL were mapped on Chromosome 1 and 2 with logP 13.2 and 10.3, respectively at 95% threshold level, with genomic regions of less than 1Mb. Significant QTL was mapped with logP 8.5 on Chromosome 7. Three suggestive QTL were also mapped on Chromosomes 3, 9 and 11 with logP of 7.5, 7.2 and 7.1, respectively. A number of candidate genes underlying the QTL are suggested. These results strongly confirmed that host susceptibility to Klebsiella pneumonia is a complex trait and controlled by multiple genetic factors and the CC population is a powerful tool for dissecting this trait.

150C
Fine mapping and candidate gene analysis of an obesity quantitative trait locus on mouse chromosome 2. Akira Ishikawa, Md. Bazlur R. Mollah. Laboratory of Animal Genetics, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan.
Obesity, a multifactorial disease, is an important predisposing factor for metabolic syndrome such as type 2 diabetes and hypertension. The genetic architecture of obesity has been poorly understood. We previously developed a congenic strain, B6.Cg-*Pbwg1*, with a 44.1-Mb genomic region derived from the Philippine wild mouse, *Mus musculus castaneus*, onto the genetic background of a common inbred strain, C57BL/6J (B6). *Pbwg1* is a major quantitative trait locus (QTL) affecting postnatal growth on mouse Chromosome 2. Our subsequent QTL analysis in an F_2 intercross population between B6.Cg-*Pbwg1* and B6 revealed that four obesity QTLs are closely linked within a small interval of the introgressed region. In this study, we developed several subcongenic strains with overlapping and non-overlapping wild-derived genomic regions from the F_2 mice. Developed subcongenic strains were phenotyped on low-fat standard and high-fat diets to fine-map the previously identified obesity QTLs. We also searched for candidate genes of the QTLs by next-generation genome DNA sequence analysis and RT-qPCR analysis. Subcongenic analysis revealed that an obesity QTL is located within an 8.8-Mb region of Chromosome 2. The

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 resistant to the high-fat diet. Next-generation sequence analysis of all exons for 27 genes residing within the 8.8-Mb region revealed many synonymous and nonsynonymous substitutions between the subcongenic and B6 strains. Among the 27 genes, four showed large differences in gene expression in liver, gonadal fat and skeletal muscle between two strains. In conclusion, the wild-derived QTL allele prevents obesity. The four differentially expressed genes may be candidates for the QTL.

151A

Wild-derived *Mus spretus* strains : a resource for genetic dissection of resistance to Plague. JEAN JAUBERT[1,2], CHARLOTTE LEBLANC[1,2], CHARLENE BLANCHET[1,2], SYLVIE GARCIA[3], ELISABETH CARNIEL[4], CHRISTIAN DEMEURE[4], ROBERT GEFFERTS[5], CLAUDIA POMMERENKE[5], KLAUS SCHUGHART[5], JEAN-JACQUES PANTHIER[1,2], XAVIER MONTAGUTELLI[1,2]. 1) Mouse Functional Genetics, Institut Pasteur, PARIS, France; 2) CNRS URA 2578, F-75015 PARIS, France; 3) Lymphocyte Population Biology, 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 (N5) females carrying a heterozygous Chr8-$^{SEG/STF}$ fragment showed statistically higher survival rate after infection than homozygous Chr8-$^{STF/STF}$ female littermates. Grafting experiments between STF and SEG strains showed that the SEG resistance phenotype could not be transferred. These results suggest that: 1) 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 peritoneal 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 datas 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. Jones[1], Leslie C. Jellen[1], Erica L. Unger[1], Christopher J. Earley[2], Lu Lu[3], Robert W. Williams[3], Xusheng Wang[3]. 1) Penn State University, University Park, PA; 2) The Johns Hopkins University, Baltimore, MD; 3) 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 from 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 linked to a significant QTL on Chromosome 2. Within this QTL is the glial high-affinity glutamate transporter (*Slc 1a2*)gene, a putative candidate, as its expression was both correlated with VMB iron and cis-regulated. We then used microarray analysis to measure VMB gene expression differences in six strains showing differential susceptibility to VMB iron loss. This revealed higher expression of *Slc 1a2* in iron deficiency in susceptible strains, further supporting *Slc 1a2* as 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 stromal cell-derived factor 1 (*Cxcl12*) and hemoglobin, beta chain (*Hbb-b1*), all key mediators of nigrostriatal dopamine functioning. The novel up-regulation of *Cxcl12* 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 USPHS Grant AG 21190.

153C

Bmi1 is regulated downstream of EGFR 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, Frederick, MD.

Stem cell-like cells are present in many forms of cancer and are important for tumor maintenance and re-establishment upon metastasis. Cell fate in normal and cancer stem cells is controlled by epigenetic changes in chromatin, however, the microenvironment is also critical for directing stem cell fate. While it is known that epigenetic regulators such as the Polycomb Group (PcG) proteins are essential for stem cell maintenance, the relationship between these cell-intrinsic factors and the cell-extrinsic factors of the microenvironment is not well understood, particularly in the context of tumorigenesis. *Bmi1*, 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 loss of the transcript resulted in decreased proliferation. Growth factor signaling studies revealed that EGFR 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 EGFR 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 *Scn2a*^{Q54} 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 SJL genomic DNA 3kb upstream and downstream from the putative *Kcnv2* coding region revealed a high degree of polymorphism between SJL and B6 strains. RACE experiments suggest multiple *Kcnv2* transcription start sites and alternative splicing with strain-dependent variation in usage. We are currently performing RNase 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. Kambhampati[1], Byron C. Jones[1], Sheila Sutti[1], Sonia A. Cavigelli[1], Christina Ragan[1], Pierre Mormède[2], Robert W. Williams[3], Lu Lu[3]. 1) Penn State University, University Park, PA; 2) Université de Toulouse, Toulouse, France; 3) University of Tennessee Health Sciences Center, Memphis Tennessee.

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, tilted cage, change in light-dark cycle, exposure to intra-species and predator urine, wet bedding, no bedding and short blasts from an air horn. 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 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 is 2h for 3 consecutive days followed by 4h on day 4. In this experiment we had a CMS group and a normal husbandry group. At the conclusion of the experiment, all animals were restrained 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 its kind to measure multiple phenotypes 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 USPHS grants AA13499 and AA17590.

156C

Maternal Separation is associated with an altered response to stress and epigenetic modifications. Rachel L. Kember, Cathy Fernandes, Emma Dempster, Leonard C. Schalkwyk, Jonathan Mill. Kings College London, London, United Kingdom.

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 increasing 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 DNA 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 7 locus for infarct volume that was also mapped for pial collateral artery density in F2 (B6xBALB/c) 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 similarly 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 single 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 mechanism from collateral circulation. Therefore, 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. Kirov[1], A. Cipollone[1], B. Willis[1], R. Araiza[1], K. Lloyd[1], D. West[1,2]. 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. To further investigate these anomalies, we quantified the relative expression of the native allele and the LacZ allele in two different HET mutants (*Snx27* and *Kmo*) by qRT-PCR. 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 to confirm that silencing may mediate reduction in the abundance of the reporter allele transcript. Supported by NIH Grants: 3U01HG004080-04S1, 3U42RR024244-03S2.

159C

Voluntary ethanol consumption in *KCNJ9* knockout mice. L. C. Kruse, A. M. Colville, L. B. Kozell, 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 both phenotypes. To assess the role of GIRK3 in ethanol consumption, we compared GIRK3 knockout (KO) and wildtype (WT) littermates (inbred C57BL/6 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 and quinine consumption. In preliminary analyses, a main effect of genotype is apparent ($F_{1,75}=4.3$, $p=0.04$), with KO mice consuming more ethanol than WT. No effect of genotype is detected for water, saccharin or quinine consumption. Multiple studies detect quantitative trait loci (QTL) 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 (Neuropsychopharm 28:932, 2003) and morphine response (Pharm 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].

160A

Animal model integration to AutDB, a genetic database for autism. Ajay Kumar, Rachna Wadhawan, Catherine Swanwick, Ravi Kollu, Saumyendra Basu, Sharmila Banerjee-Basu. MindSpec, McLean, VA.

Background: In the post-genomic era, multi-faceted research on complex disorders such as autism has generated diverse types of molecular information related to its pathogenesis. The rapid accumulation of putative candidate genes/loci for Autism Spectrum Disorders (ASD) and ASD-related animal models poses a major challenge for systematic analysis of their content. We previously created the Autism Database (AutDB) to provide a publicly available web portal for ongoing collection, manual annotation, and visualization of genes linked to ASD. Here, we describe the design, development, and integration of a new module within AutDB for ongoing collection and comprehensive cataloguing of ASD-related animal models. **Description:** As with the original AutDB, all data is extracted from published, peer-reviewed scientific literature. Animal models are annotated with a new standardized vocabulary of phenotypic terms developed by our researchers which is designed to reflect the diverse clinical manifestations of ASD. The new Animal Model module is seamlessly integrated to AutDB for dissemination of diverse information related to ASD. Animal model entries within the new module are linked to corresponding candidate genes in the original "Human Gene" module of the resource, thereby allowing for cross-modal navigation between gene models and human gene studies. Although the current release of the Animal Model module is restricted to mouse models, it was designed with an expandable framework which can easily incorporate additional species and non-genetic etiological models of autism in the future. **Conclusions:** Importantly, this modular ASD database provides a platform from which data mining, bioinformatics, and/or computational biology strategies may be adopted to develop predictive disease models that may offer further insights into the molecular underpinnings of this disorder. It also serves as a general model for disease-driven databases curating phenotypic characteristics of corresponding animal models.

161B

A Mutation in *Spef2* Results in Spermatogenesis Defects and Primary Ciliary Dyskinesia. Lance Lee[1], Anu Sironen[2], Noora Kotaja[3], Howard Mulhern[4], Todd Wyatt[5], Joseph

Sisson[5], Jacqueline Pavlik[5], Mari Miiluniemi[2], Mark Fleming[4]. 1) Sanford Children's Health Research Center, Sanford Research / University of South Dakota, Sioux Falls, SD; 2) Agrifood Research Finland, Jokioinen, Finland; 3) Department of Physiology, Institute of Biomedicine, University of Turku, Turku, Finland; 4) Department of Pathology, Children's Hospital Boston / Harvard Medical School, Boston, MA; 5) Division of Pulmonary, Critical Care, Sleep Medicine & Allergy, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE.

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 PCD and the molecular mechanisms underlying ciliary function. Mice homozygous for the mutation big giant head (*bgh*) develop phenotypes commonly associated with PCD, including hydrocephalus, sinusitis, and male infertility. Mutant males have a defect in spermatogenesis that results in short sperm tails, malformed sperm heads, and extensive death of elongating spermatids. In contrast, mutant respiratory epithelial cilia are present and have a normal ultrastructure. However, quantitative video microscopy demonstrates that *bgh* cilia beat with a reduced frequency. Using a positional cloning strategy, the *bgh* mutation was mapped to Chromosome 15, where a nonsense mutation was identified in the gene encoding Sperm flagellar protein 2 (*Spef2*). This study demonstrates that *Spef2* plays an important role in motile ciliary function and spermatogenesis, and it identifies the *bgh* mutant as a new mouse model for primary ciliary dyskinesia.

162C

Modes of Action of miR96 in Developing Inner Ear Hair Cells. Morag A. Lewis[1], Jennifer M. Hilton[1], Jing Chen[1], Cordelia Langford[1], Stijn van Dongen[2], Cei Abreu-Goodger[2], Matias Piipari[1], Anton Enright[2], Karen P. Steel[1]. 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 diminuendo, a mouse carrying an ENU-induced mutation of *miR96* in the seed region that is crucial for correct target recognition. Diminuendo homozygotes (*Dmdo/Dmdo*) have no cochlear 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 tissues. 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 neither wildtype nor mutant target sequence and are presumed to be indirect targets. Among these are *Slc26a5* (prestin), *Gfi1*, and *Ptprq*, 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 *miR96* 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.

163A

Production of optimum humanized mouse model for familial amyloidotic polyneuropathy. Zhenghua Li, Kimi 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* heterotetramers has been shown to be inhibitory to dissociation and subsequent amyloid formation. To avoid the effect of mouse TTR, we generated the humanized mouse at the mouse TtrTR gene locus using the exchangeable gene 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 gene/cDNA after insertion into a 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(-), PGK(-)IRES(+), 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 obtain the enhanced expression of a co-existing human cDNA in the mouse *Ttr* locus.

164B

The role of MYC during the initiation and progression of pancreatic ductal adenocarcinoma. Wan-Chi Lin[1], Qian Zhang[1], Rene Opavsky[1], Dominick DiMaio[1], Dean Felsher[2], Michael Hollingsworth[1], Matthias Hebrok[3], Kay-Uwe Wagner[1]. 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 cancers that arise in the pancreas are ductal 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, but 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, MYC is upregulated in a mouse model for pancreatic cancer expressing mutant KRAS. To experimentally address whether 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 less than 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.

165C

TNNI3K modulates cardiac conduction in a mouse model of cardiac sodium channelopathy. Elisabeth M. Lodder[1], Brendon P. Scicluna[1], Annalisa Milano[1], Michael W. T. Tanck[2], Connie R. Bezzina[1]. 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. Exploiting the genetic variability in F2 progeny of 129P2 and FVBN/J mice with the *Scn5a1798insD/+* mutation, we have previously mapped a quantitative trait locus (QTL) on Chromosomes 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, of these cis-eQTLs, only the *Tnni3k* transcript correlates with the PR-interval (rho=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 gene-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).

166A

Fine genetic mapping and phenotyping of a locus on the mouse Chromosome 17 involved in the control of severity of Mycobacterium tuberculosis-triggered disease. Nadezda N. Logunova, Maria Korotetskaya, Marina Kapina, Michael Averbakh, Alexander Apt. Central Institute for Tuberculosis, Moscow, Russian Federation.

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

control mapped at least three QTLs located, respectively, at the mid-distal Chromosome 3, proximal Chromosome 9 and in the vicinity of the H-2 complex at the Chromosome 17. 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 genetic background of the TB-resistant C57BL/6 mice. Assessment of the TB-related phenotypes in these mice allowed to fine-map the region of interest to the 33,9 - 34,5 Mbp interval. This region does not include "classical" MHC genes, that is, H2-E,D, L and, importantly, TNFa which bears a unique non-synonimous mutation in the I/St strain. Phenotyping of new B6.I-H2j congenic strains provided moderate differences compared to parental C57BL/6 and I/St mice. Thus, mean survival time after aerosol challenge with virulent M. tuberculosis H37Rv was: I/St =87+/-13, B.I=130=+/-24 and C57BL/6 = 231+/-24 days; CFU counts in the lungs were 5-fold higher in B6.I congenics compared to C57BL/6 mice. Novel MHC-congenics displayed more severe lung pathology compared to C57BL/6 mice: the zones of inflammation were larger and had features indicating more profound restriction of breathing function. The region of interest mapped in this study contains about 40 genes with markedly different functions, 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 accomplished cloning of 15 genes provided many polymorphisms between I/St and C57BL/6 mice.

167B

DEMONSTRATING RESISTANCE-MITIGATING EFFECT OF ARTEMISIA ANNUA PHYTOCHEMICAL BLEND WITH in-Vitro CULTURES OF PLASMODIUM FALCIPARUM AND in-vivo WITH PLASMODIUM BERGHEI ANKA IN MICE. KANGETHE N. LUCY[1], HASSANALI AHMED[2], SABAH OMAR[3]. 1) BIOCHEMISTRY, KENYA POLYNAIROBI; 2) KENYATTA UNIVERSITY; 3) KEMRI P.O BOX 54628 00200 NAIROBI.

ABSTRACT Resistance of Plasmodium falciparum to drugs such as Chloroquine and Sulfadoxine-pyrimethamine is a major problem in malaria control. Artemisinin derivatives, particularly in combination with other drugs, are thus increasingly 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 develops in their efficacy 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 parasitaemia determined to calculate the effective doses ED50 and the ED9O. 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 atp6) of sensitive and resistant parasites will be compared.

168C

Mutations in *Mcm9* expose the sensitivity of germ cells to genome instability. Yunhai Luo[1], Suzanne Hartford[1], Chen-hua Chuang[2], Christian Abratte[2], Teresa Southard[2], John Schimenti[1,2]. 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Biomedical Sciences and Center for Vertebrate Genomics, Cornell University, Ithaca, NY.

The germline 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 $Mcm9^{XG743}$, which affects genome maintenance in germ cells. *Mcm9* 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 homozygotes for the $Mcm9^{XG743}$ allele are viable. Nevertheless, considering the vulnerability of DNA to damages during replication, mutations in *Mcm9* might cause genome instability. In agreement with this expectation, $Mcm9^{XG743/XG743}$ mice displayed a high incidence of tumor formation. Metaphase spreads of mouse embryonic fibroblasts (MEFs) showed that $Mcm9^{XG743/XG743}$ MEFs had increased chromosome breaks. Apart from this, adult $Mcm9^{XG743/XG743}$ mice exhibited meiotic arrest and a lack of spermatogonia in seminiferous tubules of the testis. Female mutants had less oocytes than wild-type. Immunohistochemistry analysis of newborn gonad revealed a sex-independent germ cell depletion, suggesting the defect may originate in the primordial germ cells population. We hypothesize that the $Mcm9^{XG743}$ mutation may directly correlate to an elevated DNA damages at the cellular level and that sensitive DNA damage responses in germ cells lead to a germ cell depletion phenotype.

169A

Genetic modifiers in mouse models of motor neuron disease. Cathleen Lutz, Melissa Osborne, Don Peter Liu, Kimberly Huebsch, Leah Rae Donahue. Genetci Reouurse 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 the 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 (Burnett 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 the delta7 lines because we could measure post natal survival. We demonstrated that survival of animals on the fully congenic FVB background was shorter (averaging only 11-14 days) than that of the 005025 FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1tm1Msd/J line distributed at JAX (averaging 17-19 days) (Kariya, et al 2008). Remarkably, this same line on a C57BL/6J background failed to survive to birth. We then set out to look at the effects of genetic background on SMA animals by making congenic lines using FVB/N and C57BL/6J for a number of different SMA models. Our data show that there are dramatic changes in survival that are attributable to genetic background, suggesting the presence of modifier alleles. Presented here, are ongoing mapping studies to identify these potential loci which could prove to be useful in profiling SMA patients and identifying possible new drug targets.

170B

Segmental monosomy of a conserved interval on mouse chromosome 16 syntenic to human chromosome 21. Anna Migdalska[1], Louise Van Der Weyden[1], Ozama Ismail[1], Mark J. Arends[2], David J. Adams[1]. 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 *Lipi* to *Usp25* genes, syntenic to human region 21q11.2-21.1. This model has been developed to investigate the possible contribution of the genes located within the deleted 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 subjected our monosomy 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 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.

171C

Epigenomic analysis of dendritic cells conferring increased susceptibility to allergic asthma. Lyudmila Mikhaylova, Lester Kobzik, 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, recreating 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 asthmatic mothers develop allergic airway hyperresponsiveness and inflammation, as well as systemic features of allergy. Splenic dendritic cells (DC) from the neonates of asthmatic mothers are uniquely capable of polarizing recipients' immunity towards pro-allergic responses and confer increased allergy risk when isolated prior to allergen sensitization (Fedulov et al, 2010). This suggests that DC are functionally skewed from birth, therefore the aim of the study was to determine the underlying 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-naïve DC from the neonates of normal and asthmatic 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 about 8,000 regions (mapping to 5440 transcripts) that were hypomethylated, and about 4,400 (3547 transcripts) that were hypermethylated in asthma 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 asthmatic 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) BC5 males. Mean ± SEM. t-test * P<0.001.

	Body Wt (g)	Fat Mass (g)	Lean Mass (g)	Glucose T0 (mmol/l)	Insulin T0 (ng/ml)
Unaffected	32.3±0.6	5.5±0.4	23.1±0.4	10.7±0.3	2±0.1
Affected	46.1±0.7*	16.2±0.3*	25.7±0.4*	17.8±0.7*	7.2±0.3*

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 WTCHG, Oxford. Alignment and initial analysis of the resulting sequence identified 2 high and 1 medium confidence SNP's. Validation confirmed the two high confidence SNP's 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. Moskowitz, 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 ontogengy of atrial septal defects. We showed that avc1 is a hypomorphic mutation in intraflagellar transport protein 172 (*Ift172*), required for ciliogenesis and Hedgehog (Hh) signaling. We found that Hedgehog (Hh) signaling marked cardiac progenitors 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 septum 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 septae. We speculate that signaling from respiratory endoderm to second heart field cardiac progenitors may underlie early events in the evolution of cardiac septation.

174C

Genetic regulation of Neurexin 1 expression: An integrative cross-species analysis of schizophrenia candidate genes. Khyobeni Mozhui[1], Xusheng Wang[1], Jingchun Chen[2], Megan M. Mulligan[1], Jesse Ingles[1], Xiangning Chen[2], Lu Lu[1], Robert W. Williams[1]. 1) Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN; 2) Department of Psychiatry, Virginia Common Wealth University, Richmond, VA.
Neurexin 1 (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 autism. Expression of *Nrxn1* in hippocampus 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 genomic sequence, and expression QTL (eQTL) analysis to study the expression of this gene. We profiled a family of 72 isogenic 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 phenotypes relevant to schizophrenia and to a novel schizophrenia candidate gene with high neuronal expression (PSD3). Numerous synapse and cell signaling genes, and known schizophrenia candidates are co-expressed with Nrxn1. Cross-species co-expression analysis 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, Yuji 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 infertile 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 males 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 shower from testis around this stage. NGS produced 35 to 48 million reads from each brain sample of two wild type and two homozygote embryos. About 90 % reads with high quality score were clustered into 1.6 million unique SAGE tags. The count number of each unique tag is exactly DGE data, related to abundance of each transcript. Our preliminary analysis of DGE data by "DESeq" listed 464 tags with significant differences ($p<0.1$). Among them, 310 tags were annotated to known transcripts. Taking transcript variants into consideration, they could be merged into less than 200 tags. We verify the listed transcripts by qPCR; in parallel, we test other statistic analysis to compare reliability of DESeq.

176B

The Collaborative Cross mouse population for dissecting host susceptibility to mixed infection inducing alveolar bone loss. Aysar Nashef[1], Yaser Salaymeh[2], Ariel Shusterman[1], Richard Mott[3], Caroline Durrant[3], Ervin Weiss[1], Yael Houri-Haddad[1], Fuad Iraqi[2]. 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 initiated 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 co-challenged with Porphyromonas gingivalis and Fusobacterium nucleatum. The infection was repeated

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 CC population were susceptible ($P \leq 0.05$), while the remaining lines were resistant to alveolar bone loss. Basic bone volume of CC lines was significantly difference ($P \leq 0.05$). 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. Broad-sense heritability of the bone volume trait of naïve mice and bone loss volume of mice due to infections, based on ANOVA results, was estimated and found to be 0.84 and 0.77, respectively. The analysis of the remaining 27 lines will be completed, shortly. CC mice were genotyped with mouse SNP diversity array and QTL mapping will be performed on all the population.

177C

Immuno-genetics of Influenza A infection in mice. Tatiana Nedelko[1], Barkha Srivastava[1], Rudi Alberts[1], Heike Kolmus[1], Xavier Montagutelli[2], Klaus Schughart[1]. 1) Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Low Saxony, Germany; 2) Mouse Functional Genetics, Department of Developmental Biology, Institut Pasteur, Paris, France.

Genetic factors that are associated with increased susceptibility of the host to influenza virus infection or disease are largely unknown. Here, we studied the host response to influenza A virus infection in different mouse strains. We used several inbred mouse strains, recombinant inbred strains (BXD), Interspecific recombinant congenic strains (BcG) and backcross mice which were infected with influenza A H1N1 (PR8). We characterized in details the host response in one of the susceptible strain (DBA/2J) and in a resistant mouse strain (C57BL/6J) in terms of viral load, cytokine/chemokine profiles, lung pathology and genome-wide gene expression. In both mouse strains, chemokines, cytokines and interferon-response genes were up-regulated, indicating that the main innate immune defense pathways were activated. However, many immune response genes were up-regulated in DBA/2J much stronger than in C57BL/6J, and several immune response genes were exclusively regulated in DBA/2J. Infection of recombinant inbred strains (BXD) and F2-backcross mice (B6D2F1 x D2) is currently ongoing to identify QTLs that contribute to the difference in susceptibility 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 Polyposis in the BXH Recombinant Inbred Strains. Stephanie C. Nnadi[1], Rayneisha Watson[1], Julie Innocent[2], Beatriz Dulanto[1], Arthur M. Buchberg[1], Linda D. Siracusa[1]. 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 (FAP) develop hundreds to thousands of polyps in the colon that may become malignant if left untreated. The Apc^{Min} mouse model of FAP develops adenomatous polyps 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 polyp number, size, and location in Apc^{Min} mice. We showed that the *Pla2g2a* and *Atp5a1* genes are responsible for the protective Modifier of Min1 and 2 ($Mom1^R$ *and* $Mom2^R$) phenotypes. Hybrid progeny from a cross involving C3H/HeJ (C3H) females carrying a susceptible Mom1 locus and C57BL/6J (B6) $Apc^{Min/+}$ males showed an ~80% decrease in polyp number compared to parental B6 $Apc^{Min/+}$ mice; this data suggests the presence of additional protective alleles within the C3H genome. We utilized the BXH recombinant inbred series to uncover modifier loci that suppress polyp formation, even in the presence of susceptible $Mom1^S$ alleles. Several candidate genes were identified 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. SCN is the recipient of a Professor Fredric Reiders Ph.D. Scholarship and a Ruth L. Kirschstein National Research Service Award.

179B

A novel mutation and alternative splicing of sodium channel Scn8a ($Na_v1.6$). Janelle E. O'Brien[1], Julie Jones[1], Louise Dionne[2], Miriam Meisler[1]. 1) Department of Human Genetics, University of Michigan, Ann Arbor, MI, 48109; 2) The Jackson Laboratory, Bar Harbor, ME, 04609.

SCN8A encodes $Na_v1.6$, a neuronal voltage-gated sodium channel that is concentrated at the axon initial segment and nodes of Ranvier. $Na_v1.6$ is an important determinant of neuronal firing rates. Mice with mutations of $Na_v1.6$ 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 $Scn8a^{9J}$. Homozygous mutant mice 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 evolutionarily 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 contains an in-frame stop codon and encodes a truncated protein. $Na_v1.6$-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. Olson[1,2], Subburaman Mohan[2,3]. 1) Department of Biology, University of Redlands, Redlands, CA; 2) Musculoskeletal Disease Center, JL Pettis VA Medical Center, Loma Linda, CA; 3) Loma Linda University, Loma Linda, CA.

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 approximately 33 genes. We assessed the skeletons of these mice from 3 to 16 weeks of age using dual X-ray absorptiometry. Ts1Rhr mice were unexpectedly similar to normal controls, showing that a larger region of trisomy is necessary to recapitulate the Down syndrome phenotype. Ms1Rhr mice, in contrast, showed decreases in weight, bone mineral content, 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 I 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 chromosome 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. Otterpohl, 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, $Apc^{Min/+}$ mice, which develop intestinal tumors due to a germline mutation, provide a model to easily identify such loci. In our studies, $Apc^{Min/+}$ 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 and evaluation 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 genome 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 LG/J x SM/J F_2 and F_{34} advanced intercross lines. Clarissa C. Parker[1], Riyan Cheng[1], Greta Sokoloff[1], Jackie Lim[2], Andrew D. Skol[3], Mark A. Abney[1], Abraham A. Palmer[1,4]. 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, Section for Genetic Medicine, the University of Chicago, IL; 4) Departments of Psychiatry and

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 F_2 intercross and an F_{34} 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 median 1.5-LOD support interval was 5.55 Mb, which is a significant improvement over most existing body weight QTL mapping studies. We identified coding SNPs between LG/J and SM/J 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 LG/J and SM/J 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. Pasumarthi[1], Brian Baridon[1], Andrew Mamalis[1], Esi Djan[1], Amanda Trainor[1], Stephen M. Griffey[1], K. C. Lloyd[1], David B. West[1,2]. 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; 1 M & 1 F) 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 functional role in CV function. KRT16, a member of the keratin gene family, is expressed in epithelium, but in a pattern consistent with hyperkeratosis phenotype in humans with presumed loss-of-gene function mutation. These data collectively illustrate the value of LacZ reporter gene staining in knockout mutants to ascertain normal tissue gene expression patterns and provide functional annotation. Supported by NIH Grants: 3U01HG004080-04S1, 3U42RR024244-03S2.

184A
Genetic Heterogeneity in Commercial Outbred Mice from Taconic. Ana V. Perez[1], Jan Gray[2], Stephen Festin[2], Gerald W. Bothe[2]. 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 stocks can be the optimal model for natural populations if genetic diversity is maintained 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 therefore maximizing genetic variability. 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 strains and outbred stocks. Using a commercially available 1449 SNP MDL panel, we have assessed the genetic variation of Taconic's outbred stocks by calculating their Heterozygosity (H) and the number of polymorphisms. We tested the following stocks: ICR (IcrTac:ICR), Swiss Webster (Tac:SW), Swiss nude (NTac:NIHS-*Foxn1*^{nu}), NMRI (BomTac:NMRI), NMRI nude (BomTac:NMRI-*Foxn1*^{nu}), which are 100% Swiss-derived, and of Black Swiss (NTac:NIHBS), ICR scid (IcrTac:ICR-*Prkdc*^{scid}), NCR nude (CrTac:NCr-*Foxn1*^{nu}), and NIHS-III (Tac:NIHS-*Lyst*^{bg}*Foxn1*^{nu}*Btk*^{xid}), which are partially of Swiss origin. 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 *Foxn1*^{nu} mutation, the level of heterozygosity decreases when compared to the outbred stock without that particular allele.

185B
Milk ejection in mice LG/J x SM/J. 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 by the mother vital. In the present study we investigate the genetic architecture of variation associated with milk ejection in the first day postpartum (D1), referred to as ME, in LG/J and SM/J 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 SM/J females (39% vs. 83%, $p<0.001$), F1 and F2 generations (both showing about 95% of ME, $p<0.001$). Differences also found among SM/J 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 survivorship 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; n.s.); rather, it appears to be due to higher fat deposition, according to necropsy data (~0.77g 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 of 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 GEne Targeting 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 ubiquitous Rosa26LoxP-STOP-LoxPrtTA 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 expressing tTA in ubiquitous or tissue-specific manner. The gene, re-expressed from inducible promoter, could be silenced time-specifically by adding doxycycline to the drinking water. With the GETWISE method, it is possible to develop mouse strains where level of expression for any gene can be controlled in any tissue at any time.

187A
Mouse models for the study of SPO11 splicing isoforms in meiosis. Florencia Pratto[1], Marina Bellani[2], R. Daniel Camerini-Otero[1]. 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 SPO11 protein. This protein is widely conserved and

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. In order to understand the role of SPO11 α, we have generated a transgenic mouse carrying a BAC with a modified *Spo11* locus, in which exon 2 has been deleted, inserted ectopically into the genome. We were able to detect over-expression of SPO11 α in a wild type mouse carrying either one or seven copies of the transgene and found that over-expression of SPO11 α does not affect meiotic progression. We found that even though SPO11 α is expressed early in a *Spo11-/- Spo11α+/-* transgenic mouse, it fails to rescue the meiotic arrest characteristic of the *Spo11* knockout mouse. This supports the notion that the SPO11 β isoform is responsible for introducing the breaks. New results for a potential synapsis function for SPO11 α will be discussed.

188B

A Defect in the N-Linked Glycosylation Pathway Causes Nonsyndromic Hearing Impairment in Mice. Frank J. Probst[1], Rebecca R. Corrigan[1], Daniela del Gaudio[1], Simon S. Gao[2], Andrew P. Salinger[3], Hsiao-Yuan Tang[4], Raye L. Alford[4], John S. Oghalai[5], Monica J. Justice[1]. 1) Molec & Human Gen, Baylor Col 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.

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 in progress, as is the sequencing of DNA from a panel of patients with hearing impairment of unknown etiology. This 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.

189C

Interaction of ciliopathy proteins Cep290 and Mkks is required for structural and functional integrity of sensory neurons. Rivka A. Rachel[1], Helen L. May-Simera[2], Shobi Veleri[1], Norimoto Gotoh[1], Byung Yoon Choi[2], Jeremy McIntyre[3], Jeffery R. Martens[3], Thomas B. Friedman[2], Hemant Khanna[4], Matthew W. Kelley[2], Anand Swaroop[1]. 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 to communicate with the extracellular environment. Malfunctions in sensory cilia can lead to related and overlapping multigenic 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, deleted in the LCA mouse model rd16, appears to play an essential role in the function and survival of sensory neurons. Recently, a mutation in CEP290 has been linked to the ciliopathy Bardet-Biedl Syndrome (BBS). To identify interactions among ciliopathy genes, we investigated the interaction of the DSD domain of CEP290 with BBS proteins in a yeast two-hybrid assay. We identified the chaparonin-related protein BBS6 (MKKS) as a Cep290 interacting protein. Zebrafish morpholino knockdown of RNA for these two genes validated this specific interaction. Unexpectedly, primary sensory neuron defects in the visual, olfactory, and auditory systems arising from mutations in the DSD domain of Cep290 or from complete deletion of Mkks are partially improved in compound mutant mice. Our results demonstrate crucial interactions between Cep290 and Mkks in cilia formation and sensory function that provide new insight into neuronal development and degeneration.

190A

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 types to form adipose depots, sometimes called fat pads. There are five large depots in the mouse: the gonadal, retroperitoneal, subscapular, 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 mechanisms of 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. From this consomic line, several congenic strains have been made and are in the process of being phenotyped by necropsy. One congenic strain with breakpoints between *rs3659084* (Chr 9; 34.6 Mb) and the telomere has increased adipose depots, especially the gonadal ($F(1, 28)=7.9$, $p=.00895$), compared with littermates without the donor region. Although the adipose depot is heavier, the congenic and wild-type littermates do not differ in body weight, body length or the weight of other organs like liver, heart, or spleen ($p>0.05$). Thus *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.

191B

Molecular characterization of the translocation breakpoint in the Down syndrome mouse model, Ts65Dn. Laura Reinholdt, Yueming Ding, Griff Gilbert, Lucy Rowe, Mary Barter, Doug Hinerfeld, Leah Rae Donahue, Cathleen Lutz, Muriel Davisson. 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 congential defects and post natal disorders. 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^{16}), 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 *App*, as well as Chr 17 sequence between D17Mit19 and D17Mit58, 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.

192C

Non-trisomic homeobox gene and *Sox9* expression alters craniofacial development in a Down syndrome mouse model. Randall J. Roper[1], Cherie N. Billingsley[1], Jared R. Allen[1], Joshua D. Blazek[1], Douglas Baumann[2], Abby Newbauer[1], Andrew Darrah[1], Brad C. Long[3], Brandon Young[3], Mark Clement[4], R. W. Doerge[2]. 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

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 precursor but equivalent sized tongue as compared to normal embryos, suggesting a relative instead of actual macroglossia 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 mandible 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 Rosen[1], N. Azoulay[1], EG Griffin[1], AJ Newbury[1], L. Koganti[1], D. Truong[2], RH Fitch[2], Z. Li[3], L. Lu[3], X. Wang[3], RW Williams[3]. 1) Neurol, 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-*Tlr4*$^{lps-2/J}$/J) with a spontaneous mutation that causes bilateral nodular subcortical heterotopias and partial callosal agenesis. The wildtype (WT) BXD29/Ty was re-derived from frozen 1979 stock (BXD29/TyJ).

The mutant phenotype is unlikely to be related to the previously reported *Tlr4* mutation in this strain, as two other strains with this deletion had normal cortical morphology. Immunohistochemical staining for the subplate laminar marker *Ctgf* revealed that the heterotopias are subcortical. BrdU birth dating studies and *Cux1+* (layer 2-4 laminar marker) immunoreactivity indicate that the majority of neurons in the heterotopia are generated relatively late in gestation. *Foxp2+* 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 *Foxp2* 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. Roth[1], Richard C. Crist[1], Jeffrey R. Horner[1], Agnieszka K. Witkiewicz[2], Michael P. Lisanti[3], Gregory E. Gonye[2], Aaron Sarver[4], Arthur M. Buchberg[1]. 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 over 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 *Cav1*$^{-/-}$ mice. We are interested in the role of the loss of *Cav1* in mammary tumors because i) *CAV1* is located in a region often associated with loss of heterozygosity (LOH) in Breast cancer and ii) a dominant negative mutation in *CAV1* has been identified in 16% of Breast cancers. Thus far, we have isolated Tn insertion sequences from over 75 mammary tumors and common integration sites (CIS) have been identified. This screen will provide a database of CIS across many tumors enabling the identification of cooperating oncogenes and tumor suppressor genes critical for disease development. Each of the new genes identified individually may be useful in the diagnosis and stratification of breast cancer, and as a group may indicate novel pathways with which to target new therapeutic protocols that can be used to eliminate breast cancer. Research supported by the Ladies of Port Richmond and grants to AMB and MPL from NCI.

195C

The deleted in liver cancer 1 gene (*Dlc1*) expression during mouse embryonic development. Mohammad G. Sabbir[1,2], Michael Mowat[1,2]. 1) Manitoba Institute of Cell Biology, CancerCare Manitoba, 675 McDermot Avenue, Winnipeg, Manitoba R3E0V9, Canada; 2) Department of Biochemistry & Medical Genetics, 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 hypomorph. 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 mRNAs. Results: The *Dlc1* gene expression is strongly associated with the development of embryonic heart, the blood vasculature of the placenta and the embryo, somatigenesis, 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.

196A

Mouse models of neurodegenerative diseases. Michael Sasner, Cathy Lutz, Steve Rockwood. Genetic Resource Science, The Jackson Lab, Bar Harbor, ME.

Unhindered 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 transgenics expressing mutant human alpha-synuclein (SNCA) or PARK2 (Parkin), as well as new LRRK2 BAC transgenic lines. Targeted mutations of many relevant genes, including *Snca, Sncb, Sncg, Pink1, Park 2, 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 various "research tool" 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/grc/index.html).

Support for this project has been provided by the NIH, HHMI 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 Scherneck, Heike Vogel, Alexandra Chadt, Reinhart Kluge, Hans-Georg Joost, Annette Schurmann. Experimental Diabetology and Pharmacology, German Institute of Human Nutrition Potsdam-

Rehbruecke, 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 (*Nobq1*) 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 Mbp containing 43 genes. Interestingly, this region maps to a QTL hotspot designated as *Qrr1* (Mozzhui 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 has 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 *Nkx2-5*$^{+/-}$ mice from an F2 intercross of the inbred strains C57Bl/6 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*$^{+/-}$ offspring of mother >300 days old have 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 high 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 biologic 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. Schworer[1,2], James R. Conner[1,2], Irina I. Smirnova[1], Alexander Poltorak[1]. 1) Department of Pathology, Graduate Program in Immunology, Tufts University Sackler School of Biomedical Sciences, Boston, MA; 2) Medical Scientist Training Program, Tufts University, Boston, MA.
Control of the innate immune response requires tight regulation of its intracellular components to prevent excess inflammation. Using a phenotypic screen in the wild-derived mouse strain MOLF/Ei, we previously described that the *Why1* locus, which contains *Irak2*, promotes an earlier and more potent toll-like receptor (TLR)-mediated p38 activation and induction of IL-6 compared with the classical inbred strain C57BL/6J. A second locus, containing TIRAP, demonstrated an inhibitory epistatic interaction with the *Why1* locus. Sequence comparison revealed an N-terminal SNP evolutionarily preserved in MOLF and humans, which is mutated in B6. We propose that this SNP, along with evolutionarily preserved residues in *Irak2* between wild-derived mice and humans, support wild-derived mice as a more relevant model in which to study TLR signaling. We hypothesize that the interaction between TIRAP and IRAK2 mediates a novel regulatory mechanism of TLR activation. To assess the allelic effect of B6 and MOLF TIRAP isoforms on IL-6 production, we overexpressed each TIRAP isoform via lentiviral infection of BMDMs. TIRAP overexpression resulted in an inhibition of TLR-induced IL-6 cytokine transcription and secretion when compared to empty vector control. This result contradicts the current model for TIRAP as solely a pro-inflammatory mediator. However, we propose this result is due to the capability of TIRAP to titrate away IRAK2 from its downstream effector molecules. Measuring NFK luciferase activity in HEK293T cells transiently transfected with B6 and MOLF alleles of IRAK2 and TIRAP, we confirmed the inhibitory activity of TIRAP on downstream signaling mediated by IRAK2. These findings demonstrate the value of linkage analysis using wild-derived mice to identify important gene interactions in an evolutionarily divergent model organism.

200B
comprehensive phenotyping of mouse models. MOHAMMED SELLOUM, TANIA SORG, ABDEL AYADI, ELODIE BEDU, MARIE-FRANCE CHAMPY, ROY COMBE, BASTIEN FRICKER, HAMID MEZIANE, YANN HERAULT. INSTITUT CLINIQUE DE LA SOURIS, ILLKIRCH, France.
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 assembled a comprehensive phenotyping platform on 5 following core units: - Clinical chemistry laboratory covers biochemistry, hematology, coagulation, immunology, endocrinology exploration. - Metabolic exploration service is set up to phenotype the metabolic function, particularly in diabetes and obesity models. Currently tests are in place to analyze the body composition, glucose homeostasis, and energy expenditure, as well as the skeleto-muscular and the uro-genital systems, and the gastro-intestinal tract. - Cardiovascular and respiratory exploration. This service is set up to phenotype the function of the cardio-respiratory system. Currently tests are in place to analyze the cardiac function and anatomy, as well as the respiratory system such as in asthma models. - Behavior and nervous system. This service explores the central and peripheral nervous system, sensory systems, as well as behavior in mice. This core has developed a comprehensive tests battery to evaluate: general CNS function, affective behaviors (anxiety, depression), cognitive function, sensory thresholds and analgesia, as well as the sensory systems (visual and auditory functions) - Histology and pathology. This service provides a comprehensive histological and histopathological analysis of mutant and control mice, as well as embryology studies. The assays performed by each core unit will be presented, as well as validated flow schemes for applications in therapeutic areas.

201C
A Trim28 hypomorphic allele reveals differential requirements of KRAB zinc finger proteins during mouse embryonic development. Maho Shibata, Kristin Blauvelt, Maria Garcia-Garcia. Molecular Biology and Genetics, Cornell University, Ithaca, NY.
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 TIF1β, KAP1 or KRIP1), 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 misssense mutations in the bromodomain 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 studies in mammalian cells. Furthermore, results from genetic interaction studies suggest that TRIM28 is required as a 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 major mediator of ZFP568 functions during embryonic development.

202A
Ahnak ablation results in lean mice with enhanced insulin sensitivity. Jae Hoon Shin[1], Il Yong Kim[1], Yo Na Kim[1], Ji Won Choi[1], Seo Hyun Lee[1], Kyung Jin Roh[1,2], Mi Ra Son[4], Cheol Soo Choi[2,3], Yoon Soo Bae[4], Je Kyung Seong[1]. 1) Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, Seoul National University,

Seou, 151-742, Korea; 2) Lee Gil Ya Cancer and Diabetes Institute, Gachon University of Medicine and Science, Incheon, Korea; 3) Division of Endocrinology Gil Medical Center, Gachon University of Medicine and Science, Incheon, Korea; 4) Laboratory of Molecular Biochemistry Division of Molecular Life Sciences Ewha Woman's University, Seoul, Korea.

To elucidate the role of AHNAK on adipogenesis, we generated *Ahnak* KO mice. *Ahnak* KO mice showed growth retardation with body weight reduction due to decreased abdominal fat compared to wild type. To validate the anti-obesity role of AHNAK, HFD were challenged. The body weight of *Ahnak* KO mice on HFD showed significantly reduced with the rate of weight gain compared to wild-type littermates. The ratio of epididymal fat weight to body weight and adipocyte size significantly decreased in HFD-fed *Ahnak* KO. The expression level of transcriptional factors and enzymes leading to lipogenesis including ADIPOQ, FABP4, PPARR, FAS, and SREBF was decreased in adipose tissue from HFD-fed *Ahnak* KO mice. Next, We measured energy intake and energy expenditure using metabolic cage. Energy intake as measured by food intake was also similar between two groups. Energy expenditure were analyzed three tests including RER, energy expenditure and locomotor activity. They also showed no significant differences between two groups. The glucose infusion rate was increased in *Ahnak* KO mice than wild-type mice as a result of hyperinsulinemic-euglycemic clamps. Hepatic glucose output was decreased in *Ahnak* KO mice than wild-type mice in clamped condition but no difference in basal condition. *Ahnak* KO mice took up significantly more glucose and this glucose moved into glycolysis and glycogen synthesis. Our findings suggest that *Ahnak* KO mice are resistance to diet-induced obesity with decreased fat mass. Also, insulin-stimulated glucose uptake and metabolism were more sensitive in *Ahnak* KO mice than wild-type mice.

203B

Identification of novel QTLs Cnes4 and Cnes5 that regulate susceptibility to progressive Cryptococcus neoformans pulmonary infection. Mitra Shourian[1,2], Scott Carroll[1,3], Erin Lafferty[1,2], Kenneth Morgan[1,3,4], JC Loredo Osti[1,5], Salman Qureshi[1,2,4]. 1) Centre for the Study of Host Resistance, McGill University, Montreal, Quebec, Canada; 2) Division of Experimental Medicine, McGill University, Montreal, Quebec, Canada; 3) Department of Human Genetics, McGill University, Montreal, Quebec, Canada; 4) Department of Medicine, McGill University, Montreal, Quebec, Canada; 5) Department of Mathematics and Statistics, Memorial University, St John's, Newfoundland, Canada.

The pathogenesis Cryptococcus neoformans fungal infection is regulated primarily by host genetic factors. Human disease may be modeled using inbred mouse strains that exhibit natural variation in susceptibility to pneumonia. We have established that the C3H/HeN inbred strain presents a significantly greater lung fungal burden than the CBA/J inbred strain four weeks following intratracheal infection with C. neoformans 24067. The aim of the present study was to characterize the pulmonary immune response of susceptible C3H/HeN mice following infection and to identify genetic loci that regulate the host response to cryptococcal pneumonia. Following infection, C3H/HeN mice demonstrated a Th2 immune response with heightened airway and tissue eosinophilia, goblet cell metaplasia, and significantly higher lung *Ccl11, Il5*, and *Il13* mRNA expression relative to CBA/J mice. In contrast, resistant CBA/J mice presented greater airway and tissue neutrophilia that was associated with significantly higher lung expression of *Ifng, Cxcl10, Il17*, and *Il23* mRNA following cryptococcal infection. A genome-wide QTL analysis of 435 segregating [C3H/HeN x CBA/J] F2 (C3HCBAF2) hybrids was performed using lung fungal burden at four weeks post-infection as the quantitative trait. Single-QTL marker regression analysis identified two significant QTL on Chromosomes 1 (Cnes4, LOD = 5.79, P=0.0001) and 9 (Cnes5, LOD = 5.47, P=0.0002) that control susceptibility to cryptococcal pneumonia. These results establish additional genetic complexity of the host response to C. neoformans in association with allergic airway disease.

204C

Genetic analysis of brain size and NSC function. Debra L. Silver[1], Bill Pavan[2], Linhua Song[1]. 1) Genetics and Microbiology, Duke University Medical Center, Durham, NC; 2) Genetic Disease Research Branch, NHGRI, Bethesda, MD.

Precise control of stem cell division during development helps dictate the size, structure, and function of our organs, including the adult brain. Development of the cerebral cortex relies upon neural stem cells (NSCs), which undergo precise divisions to self-renew and produce neurons. The exact mechanisms regulating NSC division remain poorly understood. This is a significant problem as NSC dysfunction underlies many developmental brain disorders, including microcephaly (smaller brain size), and is associated with psychiatric disorders. Our goal is to elucidate the genetic mechanisms regulating NSCs and brain size. Towards this, we recently identified and characterized a new dominant mouse model of microcephaly, caused by mutation of an RNA binding protein, MAGOH. Haploinsufficiency for *Magoh* causes depletion of neural progenitors and neuronal apoptosis. *Magoh* regulates proper mitotic spindle orientation and integrity, chromosome number and genomic stability. While these studies highlighted the importance of *Magoh* in NSC division, it is currently unknown whether *Magoh* functions as part of a complex or in a non-canonical fashion to influence brain size. To date, all functions of MAGOH have been attributed to its role in the core exon junction complex (EJC). The EJC, also consisting of EIF4A3, RBM8A, and CASC3, binds spliced RNA and modulates RNA localization, translation, and decay. The role of these EJC components in mammalian development and NSCs is currently not known. In this study we evaluate the hypothesis that *Magoh* functions in a complex to regulate brain size, by determining both the expression and function of EJC components in the developing brain. Similar to *Magoh*, *Rbm8a* and *Eif4a3* are enriched in NSCs. Analysis of a gene trap mutant revealed that *Casc3* is also expressed in the developing nervous system. Through analysis of this gene trap we are evaluating how *Casc3* mutation affects embryonic and brain development. These studies will enhance our understanding of the role of RNA binding proteins in NSCs and the repertoire of genes that function in brain development.

205A

Behavioural and Molecular Changes Associated with Advanced Paternal Age: A Mouse Model. Rebecca G. Smith[1], Rachel L. Kember[1], Leo Schalkwyk[1], Joseph Buxbaum[2], Abraham Reichenberg[1], Cathy Fernandes[3], Jonathan Mill[1]. 1) Medical Research Council Social Genetic and Developmental Psychiatry Centre, King's College London, London, UK; 2) Department of Psychiatry, Mount Sinai School of Medicine, New York, New York, USA; 3) Department of Psychosis Studies, King's College London, London, UK.

Epidemiological studies have demonstrated an association between advanced paternal age and risk for several psychiatric disorders including autism and schizophrenia. In this study we used an animal model to investigate behavioural and molecular effects of advanced paternal age on the offspring. C57BL/6J females aged 2 months were mated with fathers of three different ages: 2 months to represent 'young' fathers, 10 months to represent 'old' fathers and 12 months to represent 'very old' fathers. The offspring were tested in the open field, holeboard and social recognition tasks to explore differences in their behaviours. We observed significant reductions in both social and exploratory behaviours in the offspring of old fathers. Brain and peripheral tissues were obtained from both parents and offspring and used to investigate de novo genomic changes associated with increased paternal age using Nimblegen 720K CGH microarrays. Spermatozoa undergo multiple divisions throughout the male lifespan, potentially leading to a higher incidence of de novo genomic alterations in the offspring. An alternative hypothesis is that epigenetic alterations in the germline can explain the relationship between advanced paternal age and behavioural alterations in offspring. DNA from spleen and brain were subjected to global DNA methylation analysis using the LUMA combined with pyrosequencing. To further examine advanced paternal age effects on epigenetic mechanisms, we analysed DNA methylation across selected brain expressed imprinted genes using the Sequenom EpiTYPER system. Our study provides strong evidence for deleterious effects of advancing paternal age on social and exploratory behaviour and suggests that de-novo chromosomal changes or incomplete epigenetic reprogramming are plausible explanatory factors.

206B

New genes involved in deafness from large-scale mouse screens. Karen P. Steel, Selina Pearson, Jing Chen, Neil Ingham. Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom.

Deafness is common in the human population, but for the majority of affected individuals we do not know the molecular basis, especially in the case of progressive hearing loss. New deafness genes will identify new molecules essential for normal auditory function. To identify genes underlying deafness, we have screened mice from the Sanger Institute's 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 signs of many diseases, including deafness and balance defects. To detect hearing impairment, we record Auditory Brainstem Responses (ABRs) from anaesthetised mice using pin 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 takes 20 minutes per mouse. Waveforms are analysed to give thresholds, wave amplitudes and latencies. Of the 330 genes screened so far, nine represented mutants that were 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 detected one with severe deafness (*Spns2*), three with moderate hearing impairment (*Mcph1, Lrig1, Slc25a21*), two with high-frequency hearing impairment (*Acsl4, Wbp2*) and two with mild hearing impairment (*Phf20, Fam107b*). We also found several lines with normal thresholds but reduced amplitudes or prolonged latencies of waves in the ABR waveform; these suprathreshold features may reveal new types of functional deficit. Of the eight with raised thresholds, only one would have been detected by the Preyer reflex (earflick in response to sound), emphasising the value of ABR analysis. None of these genes were expected to be involved in hearing impairment prior to screening. We are following up the most interesting mutants with more detailed analysis of the pathology in order to understand the underlying biological mechanisms. Data can be viewed at: http://www.sanger.ac.uk/mouseportal/.

207C

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 markers using in situ hybridisation and quantitative real time PCR to identify abnormalities in specification of the left-right axis. Meiotic 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^{Dom}* 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^{Dom}* 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 C3HeB/FeJ strains identified 5 loci that modified aganglionosis in *Sox10^{Dom}* 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^{Dom}* mutants. Crosses of the C57BL/6J.*Sox10^{Dom}* line with 35 distinct inbred strains were performed. Whole-mount AChE staining of GI tracts from F1 pups at P7-P10 was used to reveal the extent of total gut length (TGL) innervated and visualize regions of innervation deficit. TGL, extent of aganglionosis, and hypoganglionosis were recorded for all F1 pups. Among the strains evaluated, *Sox10^{Dom}* F1 pups from crosses with A/J were most severely affected while crosses with PWD/PhJ were the least affected. Haplotype association (HA) mapping was implemented to identify new genetic modifiers of aganglionosis. We applied EMMA to identify modifier loci for aganglionosis across the 35 strains. Significant regions were identified on Chr 1, 2, 4, 5, 12, 14 & 17 at $P<1x10^{-29}$. 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, Sushil Devkota, Jaehoon 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 expected as an E2F target gene deregulated upon *Rb* deficiency, *Pierce1* promoter did not respond to E2F1 in CDKN2Adeficient cells. 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 proteosomal 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. Swaggart[1], Ahlke Heydemann[2], Gene H. Kim[3], Jenan Holley-Cuthrell[3], Abraham A. Palmer[1], Elizabeth M. McNally[1,3]. 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 breeding the same disease causing mutation into genetically distinct background strains. Mice null for the dystrophin associated protein γ-sarcoglycan (*Sgcg* null) develop cardiomyopathy and muscular dystrophy. It was previously shown that cardiac and muscle fibrosis was enhanced in this model in the DBA/2J (*D2-Sgcg*) background and was suppressed in 129Sv/J background (*129-Sgcg*). We previously identified Ltbp4 as a modifier of skeletal muscle disease. In this study, we expanded our analysis to included not only fibrosis in the skeletal and cardiac muscle, but also cardiac function. To evaluate genetic modifiers that alter cardiac fibrosis and function in *Sgcg* null mice, we generated an F3-F4 intercross of *D2-Sgcg* and *129-Sgcg* and employed quantitative trait locus (QTL) mapping. Using a cohort of over 200 F3 and F4 mice, we analyzed multiple skeletal muscle groups and the cardiac ventricles for fibrosis by measuring hydroxyproline content to reflect collagen deposition. We also evaluated cardiac function using echocardiography. Mice were genotyped using approximately 330 markers distributed across the genome. HOP content per tissue was associated with genetic markers using QTLRel, an R based mapping program that accounts for relatedness among individuals. The Ltbp4 region of chromosome 7 was confirmed and new loci were identified.

211A

Evaluation of skeletogenesis in the Ts65Dn mouse model for Down syndrome during embryonic development. Maeve Tischbein, Clara Moore. Biology, Franklin and Marshall College, Lancaster, PA.

Down syndrome (DS) is a disorder caused by the triplication of human chromosome 21. The resulting 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 structures 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 indentifies 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 for the detection of apoptotic cells. 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 Tokuda[1], Tânia Zaverucha do Valle[1], Agnès Billecocq[2], Claudia Pommerenke[4], Robert Geffers[3], Klaus Schughart[4], Michèle Bouloy[2], Xavier Montagutelli[1], Jean-Jacques Panthier[1]. 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;

4) Experimental Mouse Genetics, Helmholtz Centre fur Infection Research, Braunschweig, Germany.

Rift 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 meningoencephalitis. 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 challenged and monitored for the survival time. QTL analysis revealed the presence of two chromosomal regions associated with time to death on chr 2 and 11: Rift Valley fever susceptible locus 1 (*Rvfs1*) and 2 (*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* (C.MBT-*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 C.MBT-*Rvfs2* macrophage supernatants than in BALB/c macrophage supernatants, suggesting increased RVFV replication in C.MBT-*Rvfs2* macrophages. Gene expression profiling in RVFV-infected macrophages from C.MBT-*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 Trachtulec[1], Petr Flachs[1], Yasuhisa Matsui[2], Jiri Forejt[1]. 1) Department of Mouse Molecular Genetics and Center for Applied Genomics, Inst. Molecular Genetics Acad. Sci. Czech Rep., Prague, Czech Republic; 2) Cell Resource Center for Biomedical Research, IDAC, Tohoku Univ., Sendai, Japan.

The Dobzhansky-Muller (DM) model of hybrid incompatibilities explains speciation by impeded epistatic interactions. These interactions result in 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 (DMIs) 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 of *Mus m. domesticus* origin (carrying the $Hst1^s$ allele) crossed to females coming from *M. m. musculus*. These hybrid males carry no sperm, while the F1 males 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 methyltransferase. The gene is necessary for successful meiosis, as the $Prdm9^{-/-}$ germ cells arrest at pachynema.

To reveal the DMIs 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 $Prdm9^{wt/-}$ on a non-hybrid background are fully fertile, the $Hst1^s$ allele is involved in DMI/s, but other DMI/s also play a role. In the reciprocal intersubspecific hybrids, the null *Hst1* allele rescued full fertility. Thus, $Hst1^s$ may participate in yet another DMI, acting in post-pachytene germ cells.

214A

Identification of *Grb10* as a Modifier of MPNST growth in the *NPcis* Mouse Model of NF1. Jessica C. A. Van Schaick[1], Christina DiFabio[1], Sandra Burkett[1], Keiko Akagi[2], Kelly H. Smith[1], Jessica Walrath[1], Robert Tuskan[1], Karlyne Reilly[1]. 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* maternal 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, potentially contributing to the decrease in *Grb10* gene expression seen in these tumors. *Grb10* is paternally imprinted in the periphery of the mouse, therefore we examined *Grb10* isoform expression and found paternal and maternal isoforms expressed in the MPNSTs. Due to these results we are examining if 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 studies identify *Grb10* as the first modifier of MPNSTs by acting in a tumor suppressive manner and demonstrate for the first time *Grb10's* causal role in the modification of cancer phenotypes.

215B

The current progress and future plan of JAPAN MOUSE CLINIC in RIKEN BRC. Shigeharu WAKANA[1], Tomohiro SUZUKI[1], Tamio FURUSE[1], Hideki KANEDA[1], Kimio KOBAYASHI[1], Ikuo MIURA[1], Hiromi MOTEGI[2], Hideaki TOKI[2], Maki INOUE[2], Osamu MINOWA[2], Tetsuo NODA[2], Kazunori WAKA[3], Nobuhiko TANAKA[3], Hiroshi MASUYA[3]. 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) have been launched in 2008, ant its aims is to evaluate comprehensive and detailed phenotypic characteristics for various mouse resources based on standardized operating procedure. We have already performed approximately 93 strains including common inbred strains, wild derived strains, genetically-engineered strains, and ENU mutant strains on JMC comprehensive phenotyping platform. From the large phenotype data of the 43 mutant or genetically modified mouse strains, we analyzed the frequency of statically 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. This means that we can detect some significant differences in almost every strain. For 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 environmental influences. Moreover we will inform the large phenotype data in public by the Pheno-pub system in our website: http://mouseclinic.brc.riken.jp/.

216C

A protein at the intersection of coat-color biology and brain disease: *Tsg101* ablation causes neurodegeneration in adult mice. Will P. Walker[1], Caroline Wee[2], Elena Sviderskaya[3], Kay-Uwe Wagner[4], Teresa M. Gunn[1]. 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.

Mice with null mutations for either the type I transmembrane protein, attractin (*Atrn*) 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 recognizes monoubiquitinated 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 (MC1R), thereby explaining the coat color defects of *Atrn* 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 *Atrn* 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 neurodegenerative pathology, 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/HeJ genome. Xiang Wang[1], Stephani Nnadi[1], Revati Koratkar[2], Karl Broman[3], Arthur Buchberg[1], Linda Siracusa[1]. 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 $Apc^{Min/+}$ mouse model. The Modifier of Min 1 (*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/HeJ (C3H) mice. By using reciprocal *Mom1* 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 $Mom1^{S/S}$ congenic

mice were intercrossed with susceptible B6 *Mom1*$^{S/S}$ mice to generate F1 hybrids; the F1 hybrid mice were then backcrossed to B6 *Min*/+ mice. The N2 offspring were either heterozygous (B6/C3H) or homozygous (B6/B6) at each locus, except *Mom1*. 487 N2 offspring were taken at 120 days of age and polyp 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 polyp multiplicity in the small intestine and colon. The results revealed several unlinked modifier loci. In addition, a subset of modifier loci appeared 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 ENU mutagenesis screen. Dawn Watkins-Chow[1], Karen Leeds[1], Raymond Mullen[1], Arturo Incao[1], Cecilia Rivas[2], William Pavan[1]. 1) Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD; 2) ES Cell and Tg Mouse Core, NHGRI, NIH, Bethesda, MD.

Melanocytes are specialized, neural crest-derived cells responsible for pigment production in the skin. Disruption in neural crest development can present as altered pigmentation in skin and/or hair and can be associated with debilitating diseases including deafness, blindness, cleft lip, congenital 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 that increase the phenotypic severity of *Sox10* haploinsufficient mice (*Sox10*^{LacZ}/+), a well-characterized mouse model of human neurocristopathies. This goal of this mutagenesis screen is to identify previously uncharacterized pathways affecting melanocyte development and to generate models relevant for dissecting human disease etiology. From analysis of 600 pedigrees in a dominant screen, we identified five 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 littermates. On a *Sox10*^{LacZ}/+ background, heterozygosity for the *Mos3* mutation causes white head spotting that is never observed in *Sox10*^{LacZ}/+ 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 melanoblasts is observed. This suggests that compared to other spotting 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 melanoma progression.

219C

Understanding the genetics of vesico-ureteric reflux using inbred mouse models. Christine L. Watt[1], Inga J. Murawski[2], Jasmine El Andalousi[2], Rita W. Maina[2], Indra R. Gupta[1,2]. 1) 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 C3H/HeJ mouse has determined that VUR is recessive 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 refluxing 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 which 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*. **Results:** All F1 hybrids generated have a 0% incidence of VUR, indicating that VUR is likely recessive in these inbred strains. The N2 progeny from these crosses exhibit the following incidences of VUR: (CBA/J x C57BL/6J)F1 x CBA/J = 30% (n=91); (DBA/J x C57BL/6J)F1 x DBA/J = 51% (n=116) and (AKR/J x C57BL/6J)F1 x AKR/J = 8% (n=112). VUR segregates independently of gender in all inbred strains. Genotyping and linkage analysis is being performed and will validate the *Vurm1* locus and shed light on potential candidate genes associated with VUR.

220A

MRC Harwell: Disease Model Discovery, Strain Archiving and Distribution and Participation in the International Mouse Phenotyping Consortium (IMPC). Tom Weaver[1], Sara Wells[1], Martin Fray[1], Niels Adams[1], Alison Walling[1], Paul Potter[2], Anne-Marie Mallon[2], Nanda Rodrigues[2], Steve Brown[2], Mark Moore[3]. 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 in order to systematically knockout every mouse gene and 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 biology, physiology and genetics.

221B

KOMP312: A Pilot Study to Create and Phenotype 312 KOMP Knockout Alleles in Mice. D. West[1,2], P. de Jong[1], K. Wasson[2], E. Engelhard[2], K. Lloyd[2]. 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 8,500 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 (IKMC). 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 7M and 7F HOM for each line. Phenotyping emphasizes LacZ expression measured histochemically 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: 3U01HG004080-04S1, 3U42RR024244-03S2.

222C

Gene expression analysis in a murine tuberculosis infection model as a systematic approach to study resistance. Esther Wilk[1], Galina Shepelkova[2], Claudia Pommerenke[1], Rudi Alberts[1], Alexander Apt[2], Klaus Schughart[1]. 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 infection 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 (I/St) strain and their backcrosses was used to analyze genome-wide gene expression patterns of whole lungs after high dose infection with M. tuberculosis H37Rv. The strongest changes of expression were found in the family of cysteine protease inhibitors for susceptible I/St mice whereas immune cell/function specific genes dominated in resistant A/Sn mice. We also performed a systematic analysis for overrepresentation of biological pathways using gene ontology terms and KEGG pathways. We could identify major differences in antigen presentation, mast cell function and B cell responses between susceptible and resistant mouse strains. In summary, these studies contribute to a better understanding of the host factors that are involved in the resistance and susceptibility to M. tuberculosis of the mammalian host. *Funded by Helmholtz-Russian Research Group (HRJRG)-116.*

223A

Ts65Dn "Down syndrome" mice extend survival of NPcis cancer model. Annan Yang, Roger H. Reeves. Dept Physiology, Johns Hopkins Univ. School of Medicine, Baltimore, MD.

Epidemiological studies report conflicting results as to whether there is a lower incidence of solid tumors in people with Down syndrome (DS). We showed that the frequency of adenomas in *Apc*^{Min} mice is significantly repressed in the trisomy (Ts65Dn) and tumor number is directly correlated with the dosage of *Ets2* tumor repressor gene (Sussan et al., Nat 451, 2008). To study if Ts65Dn protects against multiple kinds of cancer, we crossed Ts65Dn mice to the NPcis model which has only one copy of the adjacent genes, *NF1* and *Trp53*, on the same homolog and develops sarcoma, lymphoma, adrenal gland tumor (AGT) and brain tumor via LOH. Ts65Dn mice developed the same four tumor types seen in euploid NPcis, however, survival was extended significantly in Ts65Dn. This protective effect was complex, including a reduced incidence of rapidly growing sarcomas (the most common type in euploid mice) and including additional mechanisms, such as elevated apoptosis in trisomic Adrenal Tumors. There were no incidence differences for lymphoma and brain tumor, but the median survival times were longer for trisomic mice. In contrast to intestinal tumors in *Apc*^{Min}, copy number of *Ets2* had no effect on tumor incidence in NPcis on either euploid or trisomic backgrounds. It was reported recently that the microenvironment in Ts65Dn slows growth of xenografts made from long-established, highly aggressive tumor lines as a consequence of reduced angiogenesis. Using tumor cell lines newly derived from euploid sarcomas in this study. We showed reduced growth of xenografts in Ts65Dn, however, no reduction in angiogenesis was detected. Rather, the smaller size was correlated with reduced proliferation. Furthermore, we derived trisomic tumor cell lines and found trisomic xenograft was significantly smaller than euploid xenograft in euploid host. Our biological evidence shows that, as predicted from statistical analysis, trisomy is protective against multiple types of tumor both systemically and cell autonomously. The reduced cancer burden in Ts65Dn mice results from the action of multiple genes in tumorigenesis.

224B

Cyclin D3 is overexpressed in human breast cancers and compensates for the loss of Cyclin D1 during mammary tumor initiation and progression. Qian Zhang[1], Kazuhito Sakamoto[1], Chengbao Chengbao Liu[2], Aleata Triplett[1], Wan-chi Lin[1], Hallgeir Rui[2], Kay-Uwe Wagner[1]. 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-positve 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.

225C

How does mouse α-synuclein protect against human A53T mutant α-synuclein toxicity? Dan Zou, Deborah Cabin. McLaughlin Research Institute, Great Falls, MT.

α-synuclein (SNCA) is linked to sporadic and familial forms of Parkinson's disease (PD). Three missense mutations as well as duplication of unmutated *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 A53T 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 A53T mutant SNCA. We wished to determine which amino acid differences between mouse and human SNCAs 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 A53T SNCAs 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.

283C

Development of a genome-wide mutation association study (GMAS) for mutagen-induced modifying alleles. William Dove[1], James Amos-Landgraf[1], Linda Clipson[1], Richard Halberg[1], Kathleen Krentz[1], Alexandra Shedlovsky[1], Michael Newton[2], David Adams[3]. 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.Homozygosity 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 can be dubbed "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-SNPG, 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-SNPG X B6)F1 progeny sired by bona fide B6/J males.

226B

The Mouse Mutant Resource: Strain Procurement, Genetic Mapping, and Mutation Discovery for Spontaneous Mutants Arising at The Jackson Laboratory. David E. Bergstrom, Laura G. Reinholdt, Muriel T. Davisson-Fahey, Cathleen M. Lutz, Michael Sasner, Stephen A. Murray, Jeffrey Lake, Stephen Rockwood, Leah Rae Donahue, and the MMR Team. Genetic Resource Science, The Jackson Laboratory, Bar Harbor, ME.

The application of high-throughput sequencing technologies is revolutionizing the process of mutation detection. By incorporating these technologies, we in The Jackson Laboratory's Mouse Mutant Resource (JAX MMR) are extending our characterization of new spontaneous mutant strains to the point of causative gene identification. For over fifty years, the mission of the MMR (and its predecessors) has been to provide mouse models of human genetic illness to the scientific community. Through our Mouse 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 mice 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.

227C

Mouse Phenome Database (MPD). Molly A. Bogue, Terry P. Maddatu, Carol J. Bult, Stephen C. Grubb. Mouse Phenome Project, The Jackson Laboratory, Bar Harbor, ME.

The Mouse Phenome Database (MPD; phenome.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, MPD 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. MPD is also in the process of integrating gene expression datasets, which are eligible for analysis with MPD tools and correlating with phenotypic data. In addition, MPD is now providing assistance for association analysis (EMMA) in conjunction with collaborators at UCLA (E. Eskin Lab). MPD is indispensible for helping researchers select optimal strains for many research applications. MPD contains: •Data for over 600 strains of mice (inbred, RI, consomic, 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..

228A

Cryopreservation and Distribution of Mutant Mouse Models by the Sanger Mouse Genetics Project. J. Bottomley, M. Woods, H. Kundi, L. Pearson, S. Newman, H. Wardle-Jones, C. Frost, C. Ingle, J. Salisbury, J. Bussell, R. Houghton, R. Ramirez-Solis, Mouse Genetics Project. Wellcome Trust Sanger Institute, Hinxtion, Cambridge, United Kingdom.

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.

229B

The Ontological Discovery Environment. Jason A. Bubier[1], Jeremy J. Jay[1], Michael A. Langston[2], Erich J. Baker[3], Elissa J. Chesler[1]. 1) The Jackson Laboratory, Bar Harbor, ME; 2) Dept. of Elec. Eng. and Comp. Sci., The Univ. of Tenn., Knoxville, TN; 3) Sch. of Eng. and Comp. Sci., Baylor University, Waco, TX.

Over the past decade the amount of genome wide functional genomics data that has been generated has skyrocketed and many have recognized the value of an integrative analysis of these diverse studies. The Ontological Discovery Environment (ODE, www.ontologicaldiscovery.org) allows users to integrate functional genomics data across species, tissue and experimental platform. The ODE database contains over 20,000 data sets such as mouse behavioral QTL positional candidates, lists of differentially expressed genes from microarrays, functional annotations to existing ontologies, genomic regions identified from GWAS, gene set lists that others have made public and drug-related genes from the Neuroscience Information Framework database. Each set is mapped across six supported species (human, mouse, rat, fly, zebrafish and macaque) using HomoloGene families. These data sets can be stored, shared and analyzed using a suite of modular tools. Users can upload their experimentally derived gene lists and identify hierarchical similarity among the results or identify genes that are related to all or various subsets. These multi-way intersections can be further examined by overlaying them with GO and MP annotations. Given one or more genes as inputs, functionally related genes can be identified through their shared connectivity across all sets in the database. Features such as Boolean Gene Set Algebra enable the condensation of very large sets of inputs related to particular constructs, enabling a global comparison of data sets. Positional candidate lists from multiple overlapping and related QTLs can be intersected and compared to the union of all genes identified from functional studies of related biological functions. The power of ODE's integrative approach is that it leverages the availability of vast amounts of unique data from many different species, to generate novel, holistic hypotheses about gene function even for uncharacterized genes. Supported by RO1AA18776.

230C

dbVar and dbSNP: NCBI Databases of Simple and Structural Variations. Deanna M. Church, Timothy Hefferon, John Lopez, John Garner, Lon Phan, Ming Ward, Aleksey Vinokurov, Guo-Yun Yu, Hua Zhang, Dmitry Rudnev, Eugene Shektman, Rama Maiti, Douglas Hoffman, Mike Kholodov, David Shao, Christiam Camacho, Karl Sirotkin, Donna Maglott, Mike Feolo, Stephen Sherry. NCBI/NLM/NIH/DHHS, Bethesda, MD.

The National Center for Biotechnology Information (NCBI) creates and maintains a set of databases that archive, process, display and report information related to germline and somatic variants. These databases, the Database of Genomic Structural Variations (dbVar) and the Database of Single Nucleotide Polymorphisms (dbSNP), are integrated with many resources at NCBI including Gene, GeneTests, dbGaP, OMIM, PubMed, and Nucleotide. This presentation focuses on dbVar and dbSNP, summarizing current function and highlighting recent improvements. Key to both databases are the archival and processing functions. Each submission is assigned a database identifier (nssv# and ss#) based either on flanking invariant sequence or locations asserted on reference sequences. Data in dbSNP s are then processed to aggregate information from multiple submitters (assign rs#) and to calculate locations on each version of a genome and on NCBI Reference Sequences (RefSeqs). Because these stable, public accessions are citable in publications, they facilitate aggregation of information across studies. Researchers are encouraged to submit their variation data and to cite their submissions in manuscripts and on the web. Once data are accessioned, they are made available in diverse ways: Entrez searches, study-specific reports, annotation on the genome, human gene-specific displays such as Variation Viewer, and ftp transfer. Advanced search functions to allow users to find variants with specific qualities are available using our limits and advanced search functions. As we improve access to clinically relevant variation in human we are also working to provide greater access to mouse variants and models that are associated with human disease. Work at NCBI is supported by the NIH Intramural Research Program and the National Library of Medicine.

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LAMHDI, the Linking Animal Models to Human Disease Initiative. Janan T. Eppig[1], Dave Anderson[12], Anita Bandrowski[7], Bobby-Jo Breitkreutz[6], Brian Canada[11], Andrew Chatr-aryamontri[9], Keith Cheng[4], P. Michael Conn[3], Kara Dolinski[5], Mark Ellisman[8], Jeffrey S. Grethe[8], Melissa Haendel[3], Joseph Kemnitz[13], Stephen Larson[8], Maryann Martone[7], Chris Mungall[2], Carlo Torniai[3], Olga Troyanskaya[5], Mike Tyers[6], Monte Westerfield[10]. 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) UCSD Dept. of Neuroscience, San Diego, CA; 8) UCSD National Center for Microscopy and Imaging Research, San Diego, CA; 9) Univ. of Edinburgh, Edinburgh, Scotland; 1; 10) Univ. of Oregon, Institute of Neuroscience, Eugene, OR; 1; 11) Univ. of South Carolina, Columbia, SC; 1; 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, Yuichi 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 49.6Mb) for four G1 genomes. Two G1 genomes were in DBF1 (DBA2/J x C57BL/6J) and the other were C57BL/6J genetic backgrounds. By using AB SOLiD sequencer, about 85% of the targeted sequences 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 G1 in 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 Goodstadt[1], Thomas M. Keane[2], Petr Daneck[2], Richard Mott[1], David J. Adams[2], Jonathan Flint[1]. 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/EiJ mouse, 23.3% of whose genome is inaccessible, 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 heterogeneneous 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 (explaining more than 10% of phenotypic variance), we found significant enrichments for coding variants. However, these were more likely to arise from indels and structural variants than SNPs.

234A

EMMA - European Mouse Mutant Archive. Michael Hagn[1,2], Glauco Tocchini-Valentini[2], Steve Brown[2], Urban Lendahl[2], Jocelyne Demengeot[2], Ewan Birney[2], Ramiro Ramirez-Solis[2], Yann Herault[2], Lluis Montoliu[2], George Kollias[2], Thomas Rülicke[2], Radislav Sedlacek[2], Raija Soininen[2], Martin Hrabe de Angelis[1,2]. 1) Experimental Genetics, Helmholtz Zentrum München, Neuherberg, Bayern, Germany; 2) The EMMA Network, Monterotondo Scalo, Italy.

The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. A full description of these services can be viewed on the EMMA website at http://www.emmanet.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 spermatozoa 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. Takaoki Kasahara[1], Kuniya Abe[2], Tadafumi Kato[1]. 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 species such as humans. Therefore the mouse PAR tells us the genome evolution driven by the recombination. Recently we identified the mouse gene encoding melatonin synthesizing enzyme (*Hiomt*) 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 *Hiomt*, another *Sts* which was genetically expected to be on the PAR, another *Nlgn4*, 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 minisatellites 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 from129P3/J and C57BL/6ByJ mice. Cailu Lin, Natalia Bosak, Theodore Nelson, Maria Theodorides, Zakiyyah 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/6ByJ (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 (*Adip2; Adip3, Adip5, Ap7q, Bwq5, Bwq6, Nattq1, Nattq2, Sucq*). Chromosome substitution strains (CSSs) are a useful resource for positional identification of these QTLs, but extant B6 and 129 CSSs involve different substrains (129S1/SvImJ and C57BL/6J) 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/6ByJ strains using a "speed consomics" approach. During the first two backcross generations (N2 and N3) a genome scan was conducted to identify optimal breeders. 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 (*Nattq1*), Chr 2 (*Bwq5, Adip2*), Chr 7 (*Ap7q; Adip3*) and Chr 9 (*Natt2q, Bwq6, Adip5, Sucq*). Some CSS strains are now complete (C57B6/ByJ-Chr 7129P3/J C57B6/ByJ-Chr 9129P3/J) and others are nearing completion. Supported by NIH grants R01DC000882, R01AA011028 (A.A.B), R01DK058797 (D.R.R.) and the Center for Inherited Disease Research (CIDR).

237A

The Knockout Mouse Project (KOMP) Repository. Kent Lloyd[1], Pieter de Jong[2], Ray O'Neill[3]. 1) Mouse Biology Program, University of California Davis, Davis, CA; 2) Children's Hospital Oakland Research Instiutute, 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 UCD) and Regeneron. 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 infrastructure, technical resources, and faculty expertise of the UCD Mouse Biology Program and CHORI. As lead institution, UCD archives, maintains, conducts quality assurance, and distributes ES cell clones, live mouse lines, and frozen embryos and sperm, while CHORI does similarly for targeting vectors. The Repository verifies the viability, genotype, pathogen-free status, and chromosome count of KOMP ES cells, performs value-added services (conversion of ES cells into mice, reanimation of cryopreserved germplasm, germline testing, etc), and maintains an easily-navigable website (www.komp.org) where users can search, browse, and order products and services from the online catalog. Users can also register interest in genes and receive 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 FAQ, 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 (vectors, ES cell lines, mice) have been ordered and distributed to over 1200 investigators. More than 4900 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 conditional ready, will be available.

238B

Optimization of RNA-Seq to Capture the Transcriptome of a Single Blastocyst. Chiao-Ling Lo[1,2], Hyonho Chun[3], Amy C. Lossie[1,2]. 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 emergence of powerful next generation sequencing techniques, researchers can use RNA-Seq to analyze the transcriptome at single-base resolution. However, RNA-Seq requires ~200 ng of rRNA-depeleted 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, quantitating input material, cDNA amplification and multiplex library preparation. We are able to yield more than 1 µg of gel-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 protein Smoothened 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 -GLI3^{FULL} to the repressor form GLI3^{REP}, and Role 3: SUFU has roles for the regulation of neither -GLI3^{REP} 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 *Gli3* mutants including open brain and severe polydactyly, implying reduction in the GLI3 activity. Thus we analyzed the expression of the GLI proteins in T396I embryos and found that the -GLI3^{REP} protein was reduced but -GLI2^{FULL} and -GLI3^{FULL} were intact. This suggested that the Thr^{396} residue of SUFU may have specific function for the -GLI3^{REP} expression. The stability studies of -GLI3^{REP} in cultured cells also showed that the T396I SUFU protein had stabilized -GLI3^{REP} less than the wild type. Furthermore, we found that the wild-type SUFU, but not T396I, had an ability to tether the -GLI3^{REP} protein, which localized to the nucleus without SUFU, in the cytoplasm. These results suggested a novel regulatory mechanism that SUFU might tether the GLI3^{REP} protein in the cytoplasm to prevent from degradation depending on the Thr^{396} 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 inaccurate. 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 symbol changes 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 change amid order.”.

241B

Integrating "indexing" and curation at MGI to functionally annotate developmental biology. Monica McAndrews-Hill, Carol Bult, 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 mouse 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 Quosa (www.quosa.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

242C

NHGRI-funded whole exome resequencing of mouse monogenic mutants and quantitative trait loci. Jennifer L. Moran[1], Evan Mauceli[1], Snaevar Sigurdsson[1], Tim Fennell[1], Lauren Ambrogio[1], Miriam H. Meisler[2], John C. Schimenti[3], Jane Wilkinson[1], Stacey Gabriel[1], David R. Beier[4], Federica di Palma[1], Kerstin Lindblad-Toh[1]. 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.

We have established a mouse mutant resequencing initiative in order to apply next generation sequencing technology to the identification of ENU-induced and spontaneous mutations and quantitative trait loci (QTL). Funding of the initiative at the Broad Institute through The Large-Scale Genome Sequencing Program [National Human Genome Research Institute (NHGRI); NIH] has enabled pilot studies to be performed and future sequencing of approved models to be free to the mouse community. In pilot targeted resequencing studies, over 5 Mb of genomic DNA covering 565 genes was sequenced. Of 9 ENU-induced mutants in which all RefSeq exons, UTRs and promoters in non-recombinant intervals were sequenced, causal mutations were identified and validated in 5 mutants. Whole exome sequencing of coding regions throughout the human genome has been very useful for the identification of mutations in human Mendelian diseases. The recent development of a commercially available Agilent mouse exome targeted enrichment reagent enables the sequencing of 50 Mb genome-wide and over 24,000 genes. For this resequencing initiative, most models, including mapped or unmapped monogenic mutants, will be whole exome sequenced as a first pass. Those monogenic models where mutations are not discovered, as well as approved, mapped quantitative trait loci, will be sequenced by targeted sequencing of entire genomic intervals. Illumina sequence reads will be analyzed for SNPs, small insertions and deletions with the Broad's Genome Analysis Tool Kit (GATK). A Coordinating Center made up of members from the mouse community, will receive and review proposals. Applications will be accepted beginning July 1, 2011.

243A

EuroPhenome; A DCC, Annotation Pipeline and Portal for mouse phenotyping data. Hugh Morgan, Andrew Blake, Ahmad Hassan, Simon Greenaway, Hilary Gates, Rehan Shaukat, Karen Pickford, John Hancock, Steve Brown, Ann-Marie Mallon. MRC Harwell, Harwell Oxford, Oxfordshire , OX110RD United Kingdom.

The broad aim of biomedical science in the postgenomic era is to link genomic and phenotype information to allow deeper understanding of the processes leading from genomic changes to altered phenotype and disease. Essential to developing such a linkage are databases which contain information on inbred mouse strain and mutant phenotypes. The EuroPhenome project (http://www.europhenome.org) is a comprehensive resource for raw and annotated high throughput phenotyping data arising from projects such as EUMODIC. In addition to EUMODIC, EuroPhenome captures data from the Centre for Modeling Human Disease at the Toronto Centre for Phenogenomics. The phenotyping data are generated from standard operating procedures (SOPs) which are defined in the EMPReSS (http://empress.har.mrc.ac.uk) database. The progress of data capture is tracked by the Data Coordination Centre and presented on the web site to relevant members of the consortium. EuroPhenome provides users with a variety of web tools to access the data via phenotype or genotype enabling them to find mutants of interest. In addition the EuroPhenome team has developed the first phenotype annotation pipeline which can automatically annotate mouse mutants with mammalian phenotype (MP) terms by statistically analysing the raw data.

244B

The JAX Cre Repository: Improving the utility of Cre driver strains. Stephen A. Murray, Caleb Heffner, C. Herb Pratt, Michael Sasner, 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 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 Blueprint Cre Driver Project and Allen Institute for Brain Science, we are working to extensively characterize large sets of neurobiology specific Cre driver strains, leveraging Allen's high-throughput in situ hybridization capacity. However, despite the best efforts of those developing new Cre lines, the fidelity of Cre activity is not always ideal. To address this issue, we have developed a comprehensive pipeline for the characterization of Cre driver strains using a LacZ reporter strain in a wide range of tissues and at multiple time points, including both target and non-target tissues. Thus far we have completed the characterization of 29 strains, and an additional 39 are underway. Our results indicate the vast majority of Cre driver strains exhibit unexpected recombinase activity in a number of tissue types, highlighting the need for extended analysis. We have standardized our data annotation scheme to include 11 broad organ systems, 30 individual organs/structures and 89 substructures, all of which are consistent with the mouse Anatomical Dictionary. Slide-scanned images and associated annotations are published on a dedicated website and submitted to Creportal.org. This information will allow users to make informed judgments about the suitability of a particular line for their experiments, and enhance the power of large-scale mouse gene targeting projects.

245C

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 morphogenic 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 NIDCR-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.

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The Mechanisms Controlling Initiation of Genetic Recombination. Galina Petukhova[1], Fatima Smagulova[1], Kevin Brick[2], Ivan Gregoretti[2], Pavel Khil[2], R. Daniel Camerini-Otero[2]. 1) Uniformed Services University of Health Sciences, Bethesda, MD, USA; 2) National Institute of Diabetes, Digestive and Kidney Diseases, NIH, Bethesda, MD, USA.
Errors in segregation of homologous chromosomes during meiosis may result in the production of aneuploid gametes leading to reduced fertility and/or aneuploidy in offspring. Although most aneuploid conceptions are lost, aneuploidy remains the leading cause of mental retardation in humans. The orderly segregation of homologous chromosomes is ensured by homologous recombination. Both reduced recombination and abnormal location of recombination events are well-documented prerequisites of aneuploidy. The spatial distribution of recombination events is controlled at different levels and defining the mechanisms of this regulation is necessary to understand why some of the events escape this control.
Here, we map the genome-wide distribution of recombination initiation hotspots in the mouse genome. Hotspot centres are defined with approximately 200-nucleotide precision that enables analysis of the fine structural details of the preferred recombination sites. We determine that hotspots share a centrally distributed consensus motif, possess a nucleotide skew that changes polarity at the centre of hotspots, and have an intrinsic preference to be occupied by a nucleosome. Furthermore, we find that the vast majority of recombination initiation sites in mouse males are associated with testis-specific trimethylation of lysine 4 on histone H3 that is distinct from histone H3 lysine 4 trimethylation marks associated with transcription. Finally, we show that congenic strains carrying different alleles of the meiosis-specific methyl transferase *Prdm9* share no recombination hotspots, confirming the major role of *Prdm9*-mediated H3K4 trimethylation in determining the positions of recombination hotspots in mammals.

247B

Infrafrontier - The European Research Infrastructure for Phenotyping and Archiving of Model Mammalian Genomes. Michael Raess, Martin Hrabé de Angelis, The Infrafrontier Consortium. Institute of Experimental Genetics, Helmholtz Zentrum Muenchen, Neuherberg, Germany.
BACKGROUND - Mouse models are essential tools in the study of mammalian gene function and the molecular basis of human diseases. Large numbers of mutant mouse lines are being generated by the biomedical research community and by large-scale programs for mouse mutagenesis. 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, involving partners from 12 European countries and Canada. 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.

248C

The Sanger Mouse Genetics Project: large scale, high-throughput, exploratory biology of the mouse genome. Ramiro Ramirez-Solis, Ed Ryder, Richard Houghton, Joanna Bottomley, Jacqui White, Mouse Genetics Project. The Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, 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 500 different loci have been generated and nearly 300 have undergone a primary phenotypic analysis revealing a rich set of confimatory and novel genotype-phenotype relationships. A significant proportion of genes for the MGP have been selected based on hypotheses 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.

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High-throughput exploration of the genome to discover genes important for the mammalian eye. Ramiro Ramirez-Solis[1], Jacqui K. White[1], MaryAnn Mahajan[2,3], Stephen H. Tsang[3,4], David Sunter[1], Amir Assefnia[3], Vinit B. Mahajan[2,3], Sanger Mouse Genetics Project. 1) The Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom; 2) Department of Ophthalmology and Visual Sciences, The University of Iowa, Iowa City, IA; 3) Omics Laboratory, The University of Iowa, Iowa City, IA; 4) Bernard and Shirlee Brown Glaucoma Laboratory, Departments of Ophthalmology, Pathology & Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY.
The Mouse Genetics Project is a large scale, high-throughput, exploratory screen of the mouse genome to discover gene function at the organismal level in a broad set of phenotypic features of biomedical importance. The eye and its associated structures constitute one of the important areas covered by the screen. The primary screen includes assessment of the eyes presence, size, and pigmentation, palpebral closure, lacrimation, and corneal touch reflex. Further examination of the eye includes evaluation with the slit lamp and fundus analysis. Since the EUCOMM/KOMP alleles used to generate the strains carry a *lacZ* cassette under the direction of the endogenous regulatory sequences, whole mount x-gal staining of the eye is performed on heterozygous mice from all lines. If x-gal expression is positive in the eye, or if a phenotype has been detected in the tests mentioned before, histological analysis is performed to provide more cellular detail to the expression pattern or the mutant phenotype. Out of 280 lines analyzed to date, 21 mutant alleles display phenotypes in the eye. Among the affected genes are: *Arhgap25, Btbd12, Chd7, Csrp2bp, Dctn5, Dot1l, Fam134c, Git2, Gmnn, Mcph1, Mysm1, Nfkb1, Nsun2, Prkab1, Prkcz, Setmar, Slc9a8, Sparc, Spns2, and Usp42*. Inspection of The Jackson Lab phenotypic allele database reveals that only a few of these (i.e. *Csrp2bp, Sparc*) had 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.

250B

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, ECGgenie, 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 techniques. 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.

251C

The Mutant Mouse Regional Resource Centers, A national mouse archive consortium. S. Rockwood, C. Lutz, M. Sasner, L. Donahue. GRS, The Jackson Laboratory, Bar Harbor, ME.

The Mutant Mouse Regional Resource Centers are a national consortium that maintains 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 Institution 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;129S4-*Ptpn1tm2Bbk*/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 diphtheria 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 NCRR/NIH.

252A

High-throughput genotyping, quality control and gene expression analysis of targeted mutations in the mouse. Ed Ryder, Diane Gleeson, Debarati Bhattacharjee, Ross Cook, Kalpesh Jhaveri, 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 EUMODIC 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 also currently evaluating the level of reduction in gene expression in the knockout-first Tm1a allele, which relies on a strong splice acceptor site in the cassette to prevent a 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.

253B

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 strain 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 bioinformatics support for the GRC and 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.

254C

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

The availability of a 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 provided key insights into development and disease. However, it has historically been difficult for researchers to take full advantage of the wealth of information associated with clones, as data and metadata have been stored in disparate databases. The recently updated NCBI Clone DB (formerly Clone Registry) is a resource that provides integrated information for both vector-based and cell-based clones, including sequence data, map positions, gene content and distributor information (http://www.ncbi.nlm.nih.gov/clone/). For all clones, library browsers permit viewing of high-level information, while library-specific pages contain detailed information on library construction and distributors. Data is currently available for clones associated with 31 mouse genomic libraries, while data for cell-based clones from gene trap and gene targeting libraries from IKMC, MICER, IGTC and Lexicon are now being loaded. Though still under development, Entrez searches of the database will be available to support searching for specific clones by clone names, accessions, mapping position and features such as genes and STSs. Display pages for individual clones will provide an integrated view of the data stored for each clone, and link to relevant NCBI resources. Data from Clone DB is currently displayed in both the NCBI MapViewer, which permits the simultaneous viewing of annotation tracks based on different coordinate systems, and in CloneFinder, a tool that permits location-based searches for clones in a graphical interface. We will present examples of clone data and demonstrate the features of the interfaces and associated tools.

255A

Coordinated efforts for mouse phenotyping within Asia - Establishment of AMPC (Asian Mouse Phenotyping Consortium). Je Kyung Seong[1], Ki-Hoan Nam[2], Xiang Gao[3], Xiaohui Wu[4], Jeffrey Yen[5], Leo Wang[6], Si-Tse Jiang[6], Atsushi Yoshiki[7], Hisoshi Masuya[8], Shigeharu Wakana[8]. 1) Seoul National University, Seoul, Korea; 2) Biomedical Mouse Resources Center, KRIBB, Korea; 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 BioResorce Center, Japan; 8) Japan Mouse Clinic, RIKEN BioResorce Center, Japan.

The mouse has been emerging 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. Mouse phenotyping programs have been progressed in Asia, including several institutes at China, Korea, Taiwan, 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.

256B

Deciphering the genetic map of *Peromyscus*. Gabor Szalai, Paul Vrana, Adrienne Lewandowski, Jane Kenney-Hunt, Michael Felder. Peromywscus Genetic Stock Center, University of South Carolina, Columbia, SC.

Mice of the genus Peromyscus, being among the most abundant mammal in North America, are not closely related to either Rattus (rat) or Mus (mouse) and cannot hybridize with either of these species. As Peromyscus adapt readily to colony conditions and animals exhibiting traits of interest in the wild may be captured and easily reared under colony conditions, these species became widely used model organisms. To better understand murine chromosome evolution and to obtain genomic tools for gene mapping, we have assembled a medium density genetic map of Peromyscus using backcross panel to follow meiotic events in P. maniculatus (BW) x P. polionotus (PO)F1 hybrids. Genetic polymorphisms between BW and PO are easily detected as size variation in microsatellite loci and RFLPs in protein-coding genes. A total of 342 markers formed 24 linkage groups representing 23 autosomes and the X chromosome. The synteny maps between Peromyscus and the NIH model species, Mus musculus and Rattus norvegicus strongly correlated with earlier chromosome painting studies supporting the observation that the genomic organization of Peromyscus is more similar to that of the rat.

257C

Genome-wide Copy Number Variation in 162 inbred mouse strains. Jin Szatkiewicz[1], Gary Churchill[2], Fernando Pardo-Manuel de Villena[1], Patrick Sullivan[1]. 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, musculus, castaneus, and molossinus). Methods: We analyzed hybridization intensity data from 622,995 SNPs and 597,225 exon probe sets and used an integrated Hidden Markov model as implemented in PennCNV to generate an initial set of CNVs 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 mice. Our CNV catalog provides insights into the genetics of complex traits as well as genome evolution and speciation.

258A

Mouse NCBIM37 annotation in Ensembl. Amy Tang[1], Daniel Barrell[1], Susan Fairley[1], Thibaut Hourlier[1], Magali Ruffier[1], Michael Schuster[2], Simon White[1], Amonida Zadissa[1], Bronwen Aken[1], Jan-Hinnerk Vogel[1,3], Steve Searle[1], Tim Hubbard[1]. 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 trees, functional genomics and variation resources, as well as tools such as the SNP effect predictor. METHOD: The Ensembl mouse gene set was recently updated using the Ensembl automatic annotation pipeline. In addition, we merged Vega (HAVANA and Washington University, http://vega.sanger.ac.uk/Mus_musculus) manual annotation for mouse into the Ensembl annotation to provide a more comprehensive gene set for our users. RESULTS: The 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 protein-coding 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 16,864 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. Important pheromones influencing animal interactions such as aggression, comparison of the MUP gene clusters in C57BL/6J and 129 mouse strains identified significant heterogeneity. A critical component of the innate immune system, analysis of the defensins defined a species specific gene set, where expression can be restricted to specific tissue types. Similar tissue specific expression is exhibited by the takusan gene family, which are only expressed in neuronal tissues. Deficiencies in the current genome assembly combined with their restricted expression means that they are under-represented in many gene sets with only about 40 identified. However, recent analysis by our group indicates that there may be in excess of 200 takusan genes, with both coding genes and pseudogenes identified. Using new technologies, including RNA-seq and protein mass spectrometry, it should be possible to refine our annotation further and define protein-coding takusan genes on the mouse reference genome.

260C

Ensembl Genome Browser. Jana Vandrovcova[1], Ensembl Team[1,2]. 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 geneset 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 IKMC products. Information about MGI phenotypes, in-situ gene Expression (EMAGE) 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 interactive 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

A Low-density Mouse Universal Genotyping Array (MUGA). Catherine Welsh, Ryan Buus, Fernando Pardo-Manuel de Villena, Leonard McMillan. University of North Carolina - Chapel Hill, Chapel Hill, NC.

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— A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HlLtJ, 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 in an effort 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 Geneseek division at http://www.neogen.com/GeneSeek/.

262B

Sequence based characterization of structural variation in the mouse genome. Binnaz Yalcin[1], Kim Wong[2], Martin Goodson[1], Avigail Agam[1], Thomas Keane[2], David Adams[2], Jonathan Flint[1]. 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 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 evidence that SVs contribute to phenotypic variation.

263C

End-sequencing of C57BL/6N BAC clones. Atsushi Yoshiki[1], Takehide Murata[1], Takahito Yamazaki[1], Atsushi Toyoda[2], Kazuyuki Mekada[1], Satoshi Oota[1], Yukiko Yamazaki[2], Yuichi Obata[1], Kuniya Abe[1]. 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/6 substrain 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/6NCrlj 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. Bult[1], Matt Hibbs[1], Yuanfang Guan[2], Cheryl Ackert-Bicknell[1], Olga Troyanskaya[2]. 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 compendium of mouse models for human disease. MGI is also a valuable resource for the computational biology 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. Gatti[1], Cheng-Ping Fu[2], Karen L. Svenson[1], Keith Sheppard[1], Fernando Pardo Manuel de Villena[2], Leonard McMillan[2], Gary A. Churchill[1]. 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 Lenarcic[1], Gary Churchill[2], William Valdar[1]. 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, riddled 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 heritable effect.

267A

Analyzing complex high-dimensional datasets from mouse-phenotyping studies. Tonia Ludwig[1,2], Helmut Fuchs[1], Valérie Gailus-Durner[1], Martin Hrabé de Angelis[1]. 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 mutagenesis.

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 loss 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. Gennady Margolin, Pavel P. Khil, 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 Masuya[1], Terue Takatsuki[1], Mikako Saito[1], Kazunori Waki[1], Nobuhiko Tanaka[1], Kazuyuki Mekada[1], Hatsumi Nakata[1], Yuko Makita[2], Yuko Yoshica[2], Shigeharu Wakana[1], Tetsuro Toyoda[2], Atsushi Yoshiki[2]. 1) RIKEN BioResource Center, Tsukuba, Japan; 2) RIKEN BASE, 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 3,200 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,

biological taxon, MGI alleles and genes. This ensures to associate them with other metadata such as Mammalian Phenotype (MP) and Gene Ontologies to extend information network. The whole data is downloadable with in various standardized formats such as plain text, Resource Description Framework (RDF) and Web Ontology Language (OWL). Therefore, this study shows one of a good test cases for semantic-web representation of bio-resources information.

270A

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. Osadchuk[1], Mikhail S. Diakov[1], David C. Airey[2], Lu Lu[3], David W. Threadgill[4], Robert W. Williams[3]. 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/6ByJ, 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 identify a set of solutions derived from the optimal segregation model characterized by the minimal number of loci able 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.

271B

An Integrated Bayesian Hierarchical Model for Multivariate eQTL Mapping (iBMQ). Marie Pier Scott-Boyer[1], Arafat Tayeb[1], Aurelie Labbe[2], Christian F. Deschepper[1], Raphael Gottardo[3]. 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.

By studying 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-BXA recombinant inbred strains also showed that eQTL hotspots detected by iBMQ showed enrichment in genes belonging to particular GO categories.

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Whole genome sequencing: from ENU-induced mutations to mouse models of human disease. Michelle Simon, Simon Greenaway, Paul Denny, Paul Potter, Anne-Marie Mallon, Steve Brown. MRC Mammalian Genetics Unit, MRC Harwell, Harwell Science and Innovation Campus, Harwell OX11 0RD, UK.

Phenotype-driven screens after chemical mutagenesis of males with N-ethyl-N-nitrosourea (ENU) at MRC Harwell have been incredibly productive. Nevertheless, identification of the causative mutations by conventional linkage analysis and sequencing of genes in the minimal genetic interval remains a bottleneck. We have been using next generation sequencing (NGS), using the Illumina Genome Analyser 2x platform, to accelerate the process of mutation detection. A custom sequence analysis pipeline has been developed to capture and analyse causative mutations. The pipeline is based on existing packages (i.e. Bowtie, Samtools, CASAVA, nFold3, etc.) and in addition custom developed components. The first part of the pipeline is used to align reads to the C57BL/6J reference sequence, automatically identify unique variants, populate a custom sequence database and identify low and high confidence single nucleotide polymorphisms (SNPs). The second part includes assessment of the impact of putative mutations on splicing efficiency, predicted protein structure and phenotype. The EuroPhenome database at MRC Harwell captures high-throughput phenotypic data from a number of projects such as EUMODIC and in the future from the Harwell Ageing Screen. The NGS data from our pipeline will be integrated with phenotyping data in EuroPhenome and enable us to investigate the impact on mouse phenotypes and ultimately predict the likelihood of this as a model for human disease. We will present the design and implementation of the re-sequencing pipeline, the identification of unique variants and the possible impact of any SNPs on phenotype.

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Detecting novel variants with high density genotyping arrays. Hyuna Yang[1], John Didion[2], Keith Sheppard[3], Fernando Pardo-Manuel de Villena[2], Gary Churchill[3]. 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 genome 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 SNP loci. We demonstrate that these Variable INtensity Oligonucleotide probes (VINOs) result from off-target variants that interfere with hybridization. We have developed new software for genotype calling that can identify VINOs, 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 VINOs in mouse and human genotyping data on both Affymetrix and Illumina platforms. We conclude that VINOs represent a general feature of all hybridization genotyping arrays which can provide useful information beyond what is available in called genotypes.

274B

Bayesian Collaborative Cross Toolkit: a computational framework for modeling haplotype effects in multiparent lines. Zhaojun Zhang[1], Wei Wang[1], William Valdar[2]. 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 incomplete or that has a hierarchical structure. 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 that provide shrinkage estimates of actual 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 pattern and help users to better understand how genetic factors affect phenotypes.

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BioResource Proposer: the annotation and search system of mouse biological resources using molecular network information and ontologies. Kazunori Waki, Hiroshi Masuya. Technology and Development Unit for Knowledge Base of Mouse Phenotype, BioResource Center, RIKEN, Tsukuba, Ibaraki, Japan.

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.

275A

The histone demethylase KDM6A regulates Rhox gene expression in early mouse development and in ovary. Joel Berletch[1], Xinxian Deng[1], Christine Disteche[1,2]. 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 *Rhox* (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. However, 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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EPIGENETIC EFFECTS ON LOCOMOTOR ACTIVITY IN RECIPROCAL F1 MICE. Amy F. Eisener-Dorman[1], Joseph Farrington[1], Fernando Pardo-Manuel de Villena[2], Lisa M. Tarantino[1]. 1) Department of Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Department of Genetics, Lineberger Comprehensive Cancer Center, Carolina Center for Genome Science, University of North Carolina at Chapel Hill, NC.

Recent advances have revealed the complex architecture of the genome and provided new resources to explore not only genetic sequence variation but also epigenetic variation resulting from parent-of-origin effects. We have identified behavioral differences in the open field, a mouse model for anxiety, in mice from reciprocal crosses between two inbred strains, C57BL/6J (B6) and C57L/J (C57). F1 males from C57 dams (C57.B6 F1s) show a significantly higher locomotor response to novelty than F1 males from B6 dams (B6.C57 F1s). Since both B6.C57 F1s and C57.B6 F1s are genetically identical heterozygotes, each carrying one copy of each chromosome from both parental strains, we believe that the observed difference in behavior results from epigenetic differences and, specifically, parent-of-origin effects. Furthermore, the absence of this reciprocal difference in females suggests that the behavioral difference may be attributable to a locus, or loci, on the X chromosome. Parent-of-origin effects are often attributable to differential methylation of genes. Current technologies, including the newly-released Mouse Diversity Array (MDA), now allow for genome-wide assessment of methylation status in an allele-specific manner. We are currently generating additional F1 animals from both reciprocal crosses, behaviorally testing the F1s in the open field and conducting genome-wide methylation 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.

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THE EFFECT OF EARLY HOUSING ENVIRONMENT ON ANXIETY, DEPRESSION AND SOCIAL MOTIVATION. Lisa M. Tarantino[1], Sheryl S. Moy[1], Darla R. Miller[2], Patrick F. Sullivan[1,2], Fernando Pardo-Manuel de Villena[2]. 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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The Y chromosome influences experimental allergic encephalomyelitis susceptibility in male and female C57BL/6J Y chromosome consomic mice. Laure Case[1], Emma Wall[1], Naresha Saligrama[1], Elizabeth Blankenhorn[2], Cory Teuscher[1]. 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.

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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.

280C

Structural destabilization of Y chromosomes in interspecific backcrosses is consistent with variations in epigenetic modifications. Ferez S. Nallaseth[1,2], Z. Sheng Guo[2,3]. 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 (Y^{Pos}) from *Mus domesticus poschiavinus* 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 Y^{Pos}-linked repeated sequences of High Copy (HC or XY^{Pos}) female littermates 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 pI and location of these peptides can be expected to affect the stability of the DNA duplex. Denaturation of restricted genomic DNAs from HC siblings under conditions of constant temperature of 56^0C but incremental pH, identified Y^{Pos} 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 Y^{Pos} chromosome. Strain dependent variations in modifications could affect duplex stability and XY heteroduplex formation, which on resolution, lead to uncoupling of Y^{Pos} chromosome linkage.

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Genome-wide identification of maternal imprinting control regions by MeDIP-seq. Reiner Schulz[1], Charlotte Proudhon[2], Rachel Duffié[2], Guillermo Carbajosa[3], Michelle Holland[3], Vardhman Rakyan[3], Deborah Bourc'his[2]. 1) King's College London, Medical and Molecular Genetics, UK; 2) Institut Curie, UMR3215/Inserm U934, Paris, France; 3) Queen Mary University, Blizard 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 $Dnmt3l^{+/-}$ 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*/*Zdbf2* 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.

282B

BioResource Proposer: the annotation and search system of mouse biological resources using molecular network information and ontologies. Kazunori Waki, Hiroshi Masuya. Technology and Development Unit for Knowledge Base of Mouse Phenotype, BioResource Center, RIKEN, Tsukuba, Ibaraki, Japan.

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