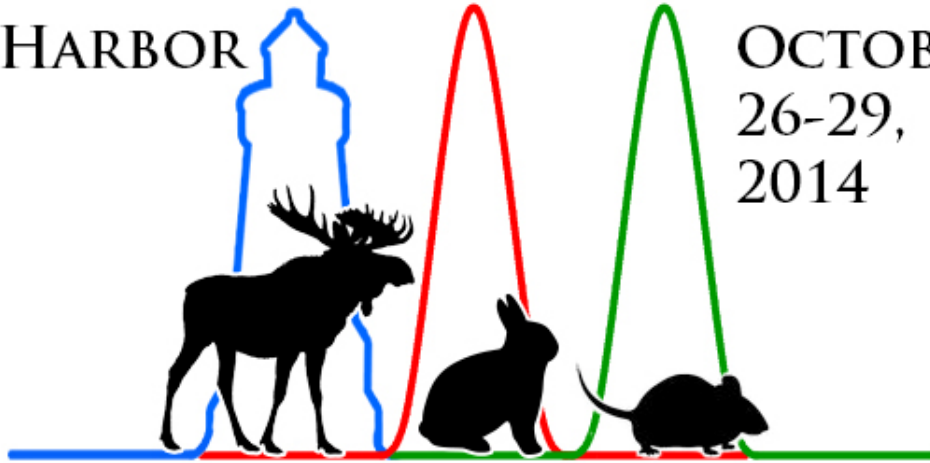

FINAL PROGRAM

BAR HARBOR, MAINE USA

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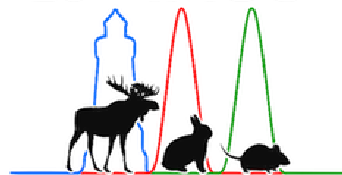
The 28th International Mammalian Genome Conference

Bar Harbor, Maine, USA
October 26-29, 2014

Final Program



Saturday, 25th October 2014		
14.00 – 18.00	Secretariat Meeting	Rockefeller Boardroom, Bar Harbor Club

Sunday, 26th October 2014		
08.00 – 20.00	Registration Open	Club Foyer, Bar Harbor Club
08.30 -	Exhibition space open to display posters	McMurtry Ballroom/Club Dining Room
09.00 – 12.30	Bioinformatics Workshop (to include mid-morning tea/coffee, but lunch is NOT included)	Stotesbury Ballroom, Bar Harbor Club
12.45	Shuttle starts running to Jackson Laboratory	In front of the Bar Harbor Club
13.30 – 18.00	Trainee Conference (Lunch NOT included)	Auditorium, **Jackson Laboratory**

13.30 SO-01: A T to C mutation in the conserved spacer region in one of the LoxP sites inhibits the Cre recombinase activity *in vivo* but not *ex vivo*

13.45 SO-02: A High-Fat, Low-Carbohydrate Ketogenic Diet Improves Aerobic Endurance in a Sex- and Strain-Dependent Fashion

14.00 SO-03: Mapping Quantitative Trait Loci with Genotyping Microarray Intensities

14.15 SO-04: Investigating and deriving induced pluripotent stem cells from the non-permissive NOD/ShLtJ and WSB/EiJ strains

14.30 SO-05: Genome-wide association study of behavior in an advanced intercross line of mice

14.45 SO-06: Network Detection for Susceptibility Genes Predisposing to Intestinal and Colon Cancers

15.00 SO-07:Identifying Key Driver Events in the C3H-*Chaos3* Mouse Model for Breast Cancer

15.15 SO-08: Analysis of the host response to influenza A virus infection in the Collaborative Cross founder strain

15.30 – 15.45 Break

15.45 SO-09: Genome evolution by duplication and differential loss in *Mus musculus*

16.00 SO-10: Mapping of QTL modifying βh1 expression in *Nan* F2 and Diversity Outbred (DO) mice

16.15 SO-11:The genetic basis of morphological variation in the skull of the gray short-tailed opossum, *Monodelphis domestica*

16.30 SO-12: Quantifying allele-specific alternative splicing in personalized genomes using EM algorithm

16.45 SO-13: High-density lipoproteins mediate microRNA intercellular communication in type 2 diabetes

17.00 SO-14: Evaluating the ENU landscape and its impact on ageing phenotypes

17.15 SO-15: Mapping host-pathogen genetic interactions to understand TB pathogenesis

17.30 SO-16: Spontaneous 8bp deletion in *Nbeal2* recapitulates the grey platelet syndrome in mice

18.30 – 21.00	Welcome Reception	Pool House Dining Room

Monday, 27th October 2014

07.30 – 09.00	Breakfast	Club Dining Room
08.00 – 18.30	Registration Open	Club Foyer
09.00 – 09.15	Opening Greetings	Stotesbury Ballroom
09.15 – 10.45	Plenary Session: **Comparative genomics, Population genetics & Evolution**	Stotesbury Ballroom
9.15	O-01: Genetic Architecture of Meiotic Drive: Mapping Genes that Distort Random Chromosome Segregation in Female Meiosis	
9.30	O-02: PRDM9 drives the evolution of recombination hotspots	
9.45	O-03: Estrous cycle: a transcriptomic analysis of 4 regions of the murine brain	
10.00	O-04: Integration of Mouse ENCODE Data with mRNA Expression QTL Data	
10.15	O-05: A new look at the genetic basis of F1 hybrid sterility of mouse inter-subspecific hybrids	
10.30	O-06: Genetic regulatory variation between inbred mouse strains	
10.45 – 11.15	Break	Club Dining Room
11.15 – 13.00	Plenary Session: **Human Disease Models I**	Stotesbury Ballroom
11.15	O-07: *Zic2* mutation causes holoprosencephaly via disruption of NODAL signalling	
11.30	O-08: A role for AMP Deaminase in malaria resistance	
11.45	O-09: A missense, gain-of-function mutation in *Tmem98* causes Bochdalek congenital diaphragmatic hernia	
12.00	O-10: Consequences of the Loss of Cholesterol Synthesis in the CNS by Inactivation of the Cholesterologenic Enzyme NSDHL are Developmental-Stage and Cell Type Specific	
12.15	O-11: Postsymptomatic rescue of clinically relevant phenotypes in a new mouse model of Spinal Muscular Atrophy	
12.30	O-12: Using human GWAS data to dissect metabolic traits in the Diversity Outbred mouse population	
12.45	O-13: Comparison of Tumorigenesis in Mouse Models of Metastatic Colorectal Cancer	
13.00 – 14.00	Lunch	Pool House Dining Room
13.00 – 14.00	Editorial Board Lunch	Pool House 2nd floor Board Room
14.00 – 16.00	Exhibition & Posters (Odd)	McMurtry Ballroom/Club Dining Room
14.30 – 16.00	MGI Workshop (optional)	Stotesbury Ballroom
16.00 – 17.30	Plenary Session: **Human Disease models II**	Stotesbury Ballroom
16.00	O-14: A Critical Role for Cyclin D1 in Testicular Germ Cell Tumorigenesis	
16.15	O-15: QTL mapping of Electrocardiographic Parameters and Cardiac Gene Expression in the BXD Recombinant Inbred Strains: Novel Loci and Candidate Genes	
16.30	O-16: Genetic and Pharmacologic Inhibition of MTOR delays Thymic Lymphoma Formation and Decreases CDK6 Levels	
16.45	O-17: A reciprocal translocation disrupts *Auts2* gene expression in a novel mutant strain.	
17.00	O-18: Practical management of laboratory research mice used to model human disease	
17.15	O-19: 9.6% of mouse gene knockouts show abnormal neuroanatomy: a resource to identify genes related to intellectual disability in human	
17.30 – 18.30	**Verne Chapman Lecture**	Stotesbury Ballroom
17.30	O-20:**Verne Chapman Lecture** Bruce Beutler Automated forward genetics	
19.30 – 20.30	Systems Genetics Workshop Lecture (optional)	
	Evening free for delegates own dinner plans	

Tuesday, 28th October 2018

Time	Event	Location
07.00 – 08.30	Breakfast	Club Dining Room
08.00 – 20.00	Registration Open	Club Foyer
08.30 – 10.00	Plenary Session: **Stem Cells and Development/Aging and Adult-onset disease modeling**	Stotesbury Ballroom
8.30	O-21: Hair graying as a model to investigate the genetics of stem cell maintenance	
8.45	O-22: Kinetochore associated protein 1 is essential for germ cell development and male fertility.	
9.00	O-23: A Systems Genetics Approach Identifies CXCL14, ITGAX, and LPCAT2 as Novel Aggressive Prostate Cancer Susceptibility Genes	
9.15	O-24: Mitochondrial dysfunction and age-dependent neurodegeneration	
9.30	O-25: An ENU-induced mutation in whirlin with a novel phenotype	
9.45	O-26: Insights into Transcriptional Regulation by SOX10 from Genome-wide Profiling of Melanocyte Cistromes	
10.00 – 10.30	Break	Club Dining Room
10.30 – 12.15	Plenary Session: **Human disease models III**	Stotesbury Ballroom
10.30	O-27: Modelling rare human neurodevelopmental disorders in mice on a large scale	
10.45	O-28: Mouse models for idiosyncratic tolvaptan-induced liver injury are identified using a Collaborative Cross approach	
11.00	O-29: Systems Genetic studies of Atherosclerosis and the novel Plasma Metabolite trimethylamine N-oxide (TMAO)	
11.15	SP-01: Student Presentation #1	
11.30	SP-02: Student Presentation #2	
11.45	O-30: MGI at 25: looking back - looking forward	
12.15 – 13.15	Nomenclature Lunch Meeting	Pool House 2nd floor Board Room
12.15 – 13.15	Lunch/Mentor Lunch	Pool House Dining Room
13.15 – 14.30	Systems Genetics Workshop Hand-on (optional) or free time	Stotesbury Ballroom
14.30 – 16.30	Exhibition & Posters (Even)	McMurtry Ballroom/Club Dining Room
15.00 – 16.30	MGI Workshop (optional)	Stotesbury Ballroom
16.45 – 17.45	O-31: **Keynote Presentation:** Jeanne Lawrence Regulating the Epigenome via Chromosomal RNAs: Implications for Genome Biology and Chromosome Pathology	Stotesbury Ballroom
17.45 – 19.00	Plenary Session: **Large-scale Resources I / Trainee Presentations**	Stotesbury Ballroom
17.45	O-32: Application of the MSBWT Utility Suite to Next Generation Sequencing Datasets	
18.00	O-33: Identification of core genes and networks regulating health and disease in the nervous system: a bioinformatic approach	
18.15	O-34: SEC23B deficiency results in different phenotypes in humans and mice	
18.30	SP-03: Student Presentation #3	
18.45	SP-04: Student Presentation #4	
19.00	Evening free for delegates own dinner plans	

Wednesday, 29th October 2014

Time	Event	Location
07.00 – 08.30	Breakfast	Club Dining Room
08.00 – 18.30	Registration Open	Club Foyer
08.30 – 10.00	Plenary Session: **Human disease models IV**	Stotesbury Ballroom
8.30	O-35: Motile Ciliary Defects and Primary Ciliary Dyskinesia in Mice Lacking *Cfap54*	
8.45	O-36: A mutation in the *Gnat2* locus, associated to achromatopsia, found in common albino outbred mice	
9.00	O-37: Bioluminescence Imaging of B Cells and Intrahepatic Insulin Gene Activity under Normal and Pathological Conditions	
9.15	O-38: Age and Sex Dramatically Affect Hyperoxic Acute Lung Injury Survival in Mice	
9.30	O-39: The Role of Primary Cilia in Neural Ciliopathic Disease	
9.45	O-40: Discovering genetic modifiers of Niemann-Pick disease, type C	
10.00 – 10.30	Break (Posters must be removed by 2PM)	Club Dining Room
10.30 – 12.00	Plenary Session: **Large-scale Resources II**	Stotesbury Ballroom
10.30	O-41: Community annotation of the rat genome	
10.45	O-42: Interspecific Recombination Between Orthologous Human and Mouse BAC Clones in *E. coli*: Exploring Scalable Humanization of Cancer-Relevant Genes in the Mouse Genome	
11.00	O-43: Dissection of expression QTL in Diversity Outbred mice	
11.15	O-44: Unbiased HIGH-THROUGHPUT mouse TISSUE phenotyping REVEALS NOVEL candidate GENES for human pathologies	
11.30	O-45: Exome sequencing reveals pathogenic mutations in 84 strains of mice with Mendelian disorders	
11.45	O-46: PopulASE: A Tool for Estimation of Isoform-specific and Allele-specific Expression with RNA-seq Data from a Genetically Diverse Population	
12.00 – 13.15	Lunch	Pool House Dining Room
12.00 – 13.15	Lunchtime Secretariat Meeting	Pool House 2nd floor Board Room
13.15 – 14.45	Plenary Session: **Large-scale Resources III**	Stotesbury Ballroom
13.15	O-47: The Mouse Genomes Project: From variants to genomes	
13.30	O-48: Collaborative Cross founder strains: A comprehensive and comparative phenotypic analysis at the German Mouse Clinic	
13.45	O-49: Spontaneous Mutation Discovery in Mouse Models of Mendelian Disease: Using RNA-Seq to Identify Non-Exomic Mutations	
14.00	O-50: Collaborative Cross founder strains – Phenotyping at the German Mouse Clinic: Known and newly identified features of strain - clinical chemistry and hematology	
14.15	O-51: The Mouse Gene Expression Database (GXD)	
14.30	O-52: The JAX KOMP2 Program: functional annotation of the mammalian genome	
14.45 – 15.00	Break	Club Foyer
15.00 – 16.00	IMGS Business Meeting (ALL encouraged to attend)	Stotesbury Ballroom
16.00 – 17.15	Plenary Session: **Advances in Genome Manipulation**	Stotesbury Ballroom
16.00	O-53: Potential Driver Genes of Ovarian Carcinogenesis identified by Sleeping Beauty Mutagenesis	
16.15	O-54: The importance of gene annotation in genome manipulation	
16.30	O-55: A conditionally inducible dominant negative allele of *Sox10* for analysis of gene function in multiple neural crest lineages	
16.45	O-56: Impact of repetitive element transcriptional changes in cocaine addiction	
17.00	O-57: Allele-specific knock-down of *KRAS* mutations in cancers by using a novel alkylating Pyrrole-Imidazole Polyamide (KR12)	
19.00 –	Conference Dinner and Awards Ceremony	Stotesbury Ballroom, Bar Harbor Club

Posters

McMurtry Ballroom/Club Dining Room	
SO-01/P-01	A T to C mutation in the conserved spacer region in one of the LoxP sites inhibits the Cre recombinase activity *in vivo* but not *ex vivo*.
P-02	Neuroscience Research Tool Strains in the JAX Repository
P-03	Generation of albino C57BL/6 mice with G291T mutation in the tyrosinase gene by the CRISPR/Cas9 system
P-04	The CrePortal (www.creportal.org), a resource for conditional mutagenesis in the mouse
P-05	Detecting cellular response to drugs in mammalian cells
P-06	Applied Genome Editing with CRISPR/Cas9 Plasmid in Mice
P-07	CRISPR/Cas9 Gene Targeting of a Gene Refractory to Conventional Methods
P-08	Kallikrein 5 and kallikrein 7 double-deficient mice generated using TALENs exhibit altered epidermal barrier
P-09	Targeting specific DNA sequences by *N*-methylpyrrole and *N*-methylimidazole polyamides provides insights for the development of novel diagnostic and therapeutic drugs
P-10	The role of Large MAF Transcription Factors in Mouse Liver Reprogramming to b-like Cells
P-11	Age-related differences in behavior and neuronal morphology of Diversity Outbred mice
P-12	Generation and characterization of novel mouse models of Parkinson’s disease
P-13	Cancer Susceptibility Loci and Modifier Loci: Insights from Inbred Mouse Strains
P-14	*R2d2* is a selfish genetic element that drives recurrent selective sweeps on mouse Chromosome 2
SO-03/P-15	Mapping Quantitative Trait Loci with Genotyping Microarray Intensities
P-16	InstantGenotype: A Non-parametric Model for Genotype Inference Using Microarray Probe Intensities
SO-09/P-17	Genome evolution by duplication and differential loss in *Mus musculus*
P-18	The Y Chromosome histone demethylase KDM5D influences H3K4me3 chromatin remodeling during meiotic progression
SO-11/P-19	The genetic basis of morphological variation in the skull of the gray short-tailed opossum, *Monodelphis domestica*
P-20	Mapping metabolic traits in the Diversity Outbred mouse population: Are we there yet?
P-21	Mining the Mouse Genome Informatics (MGI) resource for mechanistic insights into disease etiology and therapeutic potential: genotypes, phenotypes, and models of human disease

SO-02/P-22	A High-Fat, Low-Carbohydrate Ketogenic Diet Improves Aerobic Endurance in a Sex- and Strain-Dependent Fashion
P-23	The spontaneous mouse mutation ebouriffe (*ebo*), associated with postnatal lethality, infertility and wavy hair, is a nonsense allele of the *Lrrc8a* (*Swell1*) gene
P-24	The Jackson Laboratory Repository Resource: Mouse Strains for Modeling Human Disease
P-25	Effect of Genetic Background and Intestinal Barrier Function in a Murine Model of Inflammatory Bowel Disease
P-26	Industrial-Scale DNA-seq analysis enabling the 99 Lives Cat Genome Project
P-27	MGI: supporting mouse research; providing translational opportunities
P-28	Screening of a genetically complex population leads to discovery of a novel polymorphic pathway contributing to Influenza A Virus infection
P-29	Modifier Gene Discovery Using a Novel ENU Mutagenesis Approach
SO-05/P-30	Genome-wide association study of behavior in an advanced intercross line of mice
P-31	Determining the Mutifactorial Basis of Sudden Unexpected Death in Epilepsy
P-32	Integrating SNPs, epigenetics and transcriptomics to better understand the inherited predisposition to breast cancer metastasis
SO-06/P-33	Network Detection for Susceptibility Genes Predisposing to Intestinal and Colon Cancers
SO-07/P-34	Identifying Key Driver Events in the C3H-*Chaos3* Mouse Model for Breast Cancer
P-35	Variability in empathic fear among 10 inbred strains of mice
SO-08/P-36	Analysis of the host response to influenza A virus infection in the Collaborative Cross founder strain
P-37	Collateral damage: Identification and characterisation of spontaneous mutations from a targeted knockout programme
P-38	Integrated analysis of the epigenetic landscape for immortalized melanocytes switching from a proliferative to HIF1A induced cellular state
P-39	An Allelic Variant of *Mtor* Associated with DNA Damage Response and Tumor Susceptibility
SO-10/P-40	Mapping of QTL modifying βh1 expression in *Nan* F2 and Diversity Outbred (DO) mice
P-41	The genetic basis for susceptibility to the acute hepatitis form of Rift Valley fever disease in MBT/Pas inbred mice
P-42	Mouse Models of Skin, Hair, and Nail Diseases in The Jackson Laboratory Mouse Mutant Resource
P-43	Using human GWAS to mine phenotypic variation in the Diversity Outbred mouse population

P-44	Exploring differential splicing and phenotypes in mouse models of human disease
P-45	Genetic Analysis of NOD Substrain Divergence
P-46	High-Resolution Genetic Mapping of Atherosclerosis in the Diversity Outbred Mouse Population
P-47	Refinement and alignment: Combined genetic mapping and whole locus sequencing for the granulosa cell tumor susceptibility 1 (*Gct1*) locus in SWR mice
SO-15/P-48	Mapping host-pathogen genetic interactions to understand TB pathogenesis
P-49	CHD7 and SOX11 Contributions to Inner Ear and Craniofacial Development
SO-16/P-50	Spontaneous 8bp deletion in *Nbeal2* recapitulates the grey platelet syndrome in mice
P-51	Human-Mouse: Disease Connection, a tool for exploring human diseases and mouse models
P-52	Partial haploinsufficiency of the mouse *Prdm9* gene
P-53	A Genetic Interaction Network Model of a Complex Neurological Disease
P-54	The Mouse Tumor Biology Database Patient Derived Xenograft web portal: *in vivo* models of human cancer
P-55	Integrating High Throughput Phenotyping Data with Curated Phenotypes in Mouse Genome Informatics (MGI)
P-56	Spontaneous Insights from The Mouse Mutant Resource: Hypermorphs, Hypomorphs, and Novel Gene Mutations
P-57	Mouse Phenome Database
P-58	Integrative Functional Genomic Analysis using GeneWeaver.
P-59	FANTOM5, a comprehensive promoter-based gene expression atlas
P-60	Methodology for the Inference of Gene Function from Phenotype Data
P-61	OncoCL-KB, a cancer cell knowledgebase
P-62	Deep genome sequencing and variation analysis of 10 inbred mouse strains
P-63	The INFRAFRONTIER Research Infrastructure and the European Mouse Mutant Archive (EMMA)
P-64	Thy1 (CD90) regulates adipogenesis and is decreased by the environmental obesogen Tetrabromobisphenol-A
P-65	Interactive matrix views to access and explore expression information in the Mouse Gene Expression Database (GXD)
P-66	Visualizing the mouse genome
P-67	Towards a high-throughput morphological analysis of embryonic lethal knockout mice

P-68	Recent Enhancements at MGI: Cluster membership, Gene Interaction, and Mutation to Gene Relationships
P-69	Representing Homologs in the Mouse Genome Informatics System
P-70	Detecting Genome Variants using msBWT
P-71	Building the First Functional Catalogue for a Mammalian Genome: Informatics for the International Mouse Phenotyping Consortium
P-72	The FaceBase Cre Driver Project: new tools for orofacial clefting research
P-73	An Update on the Distribution Center for the Collaborative Cross Population at UNC-Chapel Hill
P-74	Analysis of sleep and wake in the JAX KOMP2 phenotyping pipeline using a non-invasive, high-throughput piezoelectric system
SO-12/P-75	Quantifying allele-specific alternative splicing in personalized genomes using EM algorithm
P-76	Mining MGI Data Using MouseMine
P-77	Looking for Mice in All the Wrong Places? Try The International Mouse Strain Resource (IMSR)-- the Place to Go to Find the Right Mouse
SO-13/P-78	High-density lipoproteins mediate microRNA intercellular communication in type 2 diabetes
P-79	High-Throughput Mouse Phenotyping will uncover new insights into Immune Mechanisms
P-80	Keeping up with the mouse reference genome assembly
P-81	Czech Centre for Phenogenomics: new research infrastructure for production of mouse models and their phenotyping
SO-14/P-82	Evaluating the ENU landscape and its impact on ageing phenotypes
P-83	Mutant Mouse Resource and Research Centers at The Jackson Laboratory
P-84	*Zic3* is a novel, *in vivo* inhibitor of β-catenin/TCF mediated transcription
P-85	Transcriptional deconvolution of meiotic substages using RNA-seq and cytological analysis of the first wave of spermatogenesis in the mouse
P-86	Embryonic stem cells from mouse models of aneuploidy syndromes
P-87	A Mouse Embryonic Phenotyping Platform for Understanding Developmental Disorders
SO-04/P-88	Investigating and deriving induced pluripotent stem cells from the non-permissive NOD/ShLtJ and WSB/EiJ strains
P-89	Evaluation of carry-over effects in the high-throughput JAX KOMP2 adult phenotyping pipeline

Abstracts:

Student and Post-Doc Presentations

SO-01/P-01: A T to C mutation in the conserved spacer region in one of the loxP sites inhibits the cre recombinase activity *in vivo* but not *ex vivo*.

Tania D Arguello[*1], and Carlos T Moraes[2]

[1]Human Genetics and Genomics Graduate Program, University of Miami, Miller School of Medicine, Miami, FL 33136, USA
[2]Department of Neurology, University of Miami Miller School of Medicine, Miami, Florida 33133

The cre/loxP recombination system is a valuable tool used to generate tissue specific knockout mice models and study gene function. The deletion of a region of interest flanked by two loxP sites is accomplished by the recombinase (cre) enzyme; this reaction involves binding of cre to the inverted repeat segments of two loxP sites and recognition of a conserved TA sequence in the central spacer region "ATAACTTCGTATA -NNNTANNN-TATACGAAGTTAT. *In vivo*, we found that a single T to C mutation at position -1 of the spacer (resulting in a TA to CA change in the central spacer region) in only one loxP site, inhibits completely the recombination reaction in two conditional mouse models (brain and muscle) generated using a cre transgene under the control of two tissue-specific promoters: calcium/calmodulin-dependent kinase II alpha (Tg(Camk2a-cre)3Szi or Camk2a-cre) and myosin light polypeptide 1 ($Myl1^{tm2(cre)Sjb}$ Myl1-cre). Surprisingly, transient transfection of a plasmid encoding cre in dermal fibroblasts derived from the same mutant floxed (loxP/loxP) mice line, generated recombinants and successfully deleted the region of interest. This study demonstrates the sequence specificity required *in vivo* and the possibility of bypassing this limitation by expressing very high levels of cre recombinase *ex vivo*.

SO-02/P-22: A High-Fat, Low-Carbohydrate Ketogenic Diet Improves Aerobic Endurance in a Sex- and Strain-Dependent Fashion

William Barrington[*1], Daniel Pomp[2], and David W Threadgill[3]

[1]North Carolina State University, Genetics, Raleigh, NC
[2]University of North Carolina Chapel Hill, Genetics, Chapel Hill, NC
[3]Texas A&M University, Molecular and Cellular Medicine, College Station, Texas

While it is understood that diet can significantly impact physiology and health, it is unclear how genetic background and sex interact with diet to produce different responses. To address this gap in knowledge our study examined the physiological effects of six diets in each sex of four inbred mouse strains. The diets represent current Western diet, traditional Mediterranean diet, traditional Japanese diet, a hunter-gatherer diet, and a ketogenic diet. The four inbred mouse strains, A/J, C57BL/6J, FVB/NJ, and NOD/ShiltJ were selected based upon their known susceptibilities to cancer and other diseases. We have identified that diet impacts aerobic endurance in a forced exercise treadmill test. Female mice of all strains tested have an aerobic endurance boost when fed a high-fat, low-carbohydrate ketogenic diet. Of the male mice, only the FVB strain showed an aerobic benefit from the diet. Paradoxically, FVB mice were able to run farthest while also experiencing the highest levels of adiposity when fed a ketogenic diet. Currently, we are working to identify how sex influences response to a ketogenic diet and determine the genetic causes underlying the FVB strain's unusual response to the diet. By doing so we hope to understand how humans respond differently to a low-carbohydrate diet and determine which individuals might experience an improvement in aerobic endurance with a low-carbohydrate diet.

SO-03/P-15: Mapping Quantitative Trait Loci with Genotyping Microarray Intensities

Chen–Ping Fu[*], and Leonard McMillan

UNC-Chapel Hill, Department of Computer Science

Many methods have been developed for mapping quantitative trait loci (QTLs) using microarrays. Traditional methods for QTL mapping rely on the assumption that biallelic genotype calls from microarrays represent the complete genetic variation at a marker. In reality, the process of converting microarray intensities to discrete genotype calls results in the loss of marker information on other variations nearby the target SNP, such as copy numbers, deletions, or off-target SNPs involving the marker sequence.

We have developed a novel approach to QTL mapping that directly uses microarray marker intensities. Our method scans for markers where the intensity distances between pairs of samples are correlated with the quantitative phenotype differences between pairs of samples. The presence of such markers indicates that samples which are genetically close together in the region also share similar phenotype values, suggesting the presence of a QTL. The significance of putative QTLs is then assessed through permutation testing. By directly incorporating genotype intensities, our method eliminates many intermediate processes such as genotype calling or ancestry inference that may introduce uncertainty or data loss.

We tested our method on synthetic phenotype data of mice genotyped with the 78K-marker MegaMUGA array, and our results compared favorably to those of R/qtl, a well-established QTL mapping package. In addition, we used our method to map the binary albino trait in inbred and backcrossed mice to the *Tyr* gene on Chromosome 7, and we also verified several QTLs found to affect colitis-related traits from a previous mouse study. We also plan to test the incorporation of covariates through a modification of our current method.

SO-04/P-88: Investigating and deriving induced pluripotent stem cells from the non-permissive NOD/ShLtJ and WSB/EiJ strains

Tiffany A Garbutt*[1], David L Aylor[1], and David W Threadgill[2]

[1]North Carolina State University, Department of Biological Sciences, Program in Genetics
[2]Texas A&M University, Department of Molecular and Cellular Medicine, College of Medicine.

Induced pluripotent stem cells (iPSCs) are derived from somatic reprogramming and have become a potential alternative to true embryonic stem cells (ESCs) in medical and general research. Non-permissive mouse strains cannot form ESCs or iPSCs under standard conditions and form developmentally primed epiblast stem cell (EpiSC)-like colonies with limited cell and research potential. We generated fibroblast-derived ESC-like iPSCs from six of the eight parental strains of the recombinant inbred Collaborative Cross (CC) mouse genetic reference population. The NOD/ShLtJ (NOD) strain, a common model for Human Type I Diabetes, and the WSB/EiJ (WSB) strain were non-permissive. We generated fibroblast-derived ESC-like iPSCs from an F1 cross between NOD and the permissive 129S1/SvImJ strain, indicating that the EpiSC-like state is recessive to the ESC-like state. A complementation test revealed that the NOD and WSB EpiSC-like iPSC phenotypes do not complement. Using a glycogen synthase kinase 3β inhibitor, a mitogen-activated protein kinase inhibitor, and varying treatment length, we derived ESC-like iPSCs and EpiSC-like iPSCs from both the NOD and WSB strains. Studies are underway using immunofluorescence staining, RNA sequencing, and DNase hypersensitivity to confirm cell state, compare genomic differences between strains, and investigate epigenetic modifications between cell states and treatments.

SO-05/P-30: Genome-wide association study of behavior in an advanced intercross line of mice

Natalia M Gonzales*[1], Shyam Gopalakrishnan[1], and Abraham A Palmer[1,2]

[1]Department of Human Genetics, University of Chicago, Chicago, IL 60637
[2]Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL 60637

Mice are one of the primary model organisms used to study behavior. We are conducting a genome-wide association study of conditioned place preference (CPP) for methamphetamine in a LG/J x SM/J advanced intercross line (AIL) of mice (Aap:LG,SM-G50-56, derived from Jmc:LG,SM-G33). CPP has been widely used to study the motivational effects of drugs of abuse in rodents. We are also using the AIL to study the genetics of prepulse inhibition (PPI) and multiple other complex traits. We are using a genotyping-by-sequencing strategy to genotype over 1,000 individuals from generations 50-56 of the LG/J x SM/J AIL. For a subset of these mice we will also measure gene expression in the striatum, hippocampus and prefrontal cortex. This data will be used to identify QTLs that regulate gene expression (eQTLs). Integrating genotype, phenotype and gene expression data is a powerful approach that will accelerate the process of gene identification and provide insight into the biological mechanisms influencing the development of drug abuse. Here we present preliminary data from individuals from generations 50-53 of the LG/J x SM/J AIL.

SO-06/P-33: Network Detection for Susceptibility Genes Predisposing to Intestinal and Colon Cancers

Dean Ihemesie*[1], Xiang Wang[1], Stephanie Nnadi[1], Revati Koratkar[2], and Linda Siracusa[1]

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Current worldwide predictions are that 2.4 million cases of colorectal cancer (CRC) will be diagnosed annually by 2035. Colorectal cancer is the second leading cause of cancer-related deaths among both men and women, and third in incidence in the U.S. A mutation in the Adenomatous Polyposis Coli (*APC*) gene is frequently the initiating factor for CRC. Mice carrying mutant *Apc* alleles have served as useful models for studying intestinal and colon tumorigenesis; in particular the Apc^{Min} mutation predisposes mice to polyposis along their intestinal tract. Although the susceptible C57BL/6J (B6) and resistant C3H/HeJ (C3) inbred strains were both bred to carry the Apc^{Min} mutation, their tumor phenotypes are vastly different. These differences are due to modifier genes that alter phenotypic manifestations of the Apc^{Min} mutation. We are testing a novel hypothesis, namely that coordinate regulation of a network of genes is responsible for tumor susceptibility. Candidate networks are identified using Ingenuity Pathway Analysis (IPA), which creates networks *de novo* based on connections drawn from the literature. We have introduced a strict criteria to this analysis, namely that a network must contain genes from all modifier loci that we had previously detected in N2 offspring from a backcross of C3B6F1 x B6 Apc^{Min}/+ mice. We screen genes within networks via quantitative real-time PCR to find those with composite expression level signatures consistent with up- or down-regulation of polyposis. This study may provide a new approach for defining networks of genes that regulate cancer susceptibility. Research supported in part by NCI grants to LDS.

SO-07/P-34: Identifying Key Driver Events in the C3H- *Chaos3* Mouse Model for Breast Cancer

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Breast cancer is a highly heterogeneous disease, comprised of several sub-types with varying genetic, genomic and histopathological features, which in turn produce diverse and sometimes unpredictable outcomes. To study cancer pathogenesis, we need to be able to recapitulate key cellular events that lead to transformation, as well as the relative order in which these events occur. Ultimately, an imperative goal of cancer research is to better understand the mechanistic causes of disease initiation and progression, and pre-clinical experimental models provide a powerful means for this investigation.

The C3HeB/FeJ-*Mcm4*^{Chaos3}/*Mcm4*^{Chaos3} (C3H-*Chaos3*) mouse has multiple characteristics that make it a highly relevant breast cancer research model. The most important of these characteristics is the genomic instability phenotype resulting from a single amino acid alteration in MCM4 [F345I], which leads to recurring chromosomal mutations that overlap significantly with that of human breast cancer. I will be exploiting this feature to determine sporadic initiating events driving mammary carcinogenesis in these mice. Specifically, I am studying the transforming potential of a recurring genomic deletion region - mouse Chr4qD3; human Chr1p36.11 – that was identified through aCGH analyses of C3H-*Chaos3* **m**ammary **t**umors (MTs), to determine whether its tumorigenic effect is cumulative or gene-specific. I am also examining the function of a potent transcription factor and known tumor suppressor gene, *Arid1a*, located within this region. *Arid1a* is deleted at a high rate across C3H- *Chaos3* MTs, and recent cancer genome sequencing studies have revealed it to be frequently mutated in a broad range of tumor types. I will be characterizing the mammary-specific role of *Arid1a* in the context of tumorigenesis, by designing a conditional knock-out mouse model for this purpose.

SO-08/P-36: Analysis of the host response to influenza A virus infection in the Collaborative Cross founder strain

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Influenza A virus poses a major health threat and caused multiple severe pandemics in the last century with millions of death. The course and outcome of an influenza A infection is influenced by viral as well as host factors. The recently established Collaborative Cross (CC) has a high genetic diversity and thus represents a well-suited mouse genetic reference population to examine genetic factors that influence host-susceptibility and resistance to influenza A infection.

Here, we present our analysis of the eight CC founder strains (A/J, C57BL/6J, 129S1/SvlmJ, NOD/ShiLtJ, NZO/HILtJ, PWK/PhJ, CAST/EiJ and WSB/EiJ) and four CC lines (OR13140, OR13067, IL16188, IL16211) after infection with the mouse-adapted virus strain influenza A/HK/01/68 (H3N2). Mice were intra-nasally infected and body weight and survival was monitored for 14 days. In addition, we characterized the host response in more detail by analyzing the hemogram of peripheral blood and determining viral loads and histopathological changes in infected lungs. We could observe large differences between the CC founder strains and the four CC lines.

SO-09/P-17: Genome evolution by duplication and differential loss in *Mus musculus*

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Segmental duplication is an important force shaping the evolution of eukaryotic genomes. Sequence redundancy transiently relieves selective pressure, allowing duplicated sequences (paralogs) to evolve specialized functions (subfunctionalization), new functions (neofunctionalization) or be reduced to ancestral copy number. Along with retrotransposition, duplication encompassing coding sequences creates and expands gene families. Evidence from diverse taxa indicates that duplication begets further duplication: segmentally-duplicated regions are hotspots for copy-number polymorphism. Importantly, differential loss of paralogs between lineages creates divergent gene repertoires which may be functionally non-equivalent. We present in detail the natural history of one such locus in *Mus musculus*, *R2d2*, which is the responder in a recently-described meiotic drive system on mouse Chromosome 2. We demonstrate that the present structure of the locus is the result of a duplication event ancestral to the divergence of *M. musculus* from *M. spretus*, followed by retrotransposition, exchange between duplicate copies, differential loss of paralogs within and between three *M. musculus* subspecies, and finally further expansion by tandem duplication in the *M. m. domesticus* lineage. The locus has an extremely low recombination rate and is highly polymorphic in both laboratory and wild mice sampled from around the globe. Using a novel alignment-free analysis of whole-genome resequencing data, we show that paralogy which is not captured in reference genome leads to the conflation of allelic and paralogous variation and spuriously-high estimates of local sequence divergence. In addition, we identify several other megabase-sized regions of the mouse genome which carry similar signatures of paralogous variation arising via duplication and differential loss between lineages. All overlap a cluster of olfactory or immune-cell receptor genes, suggesting an evolutionary basis for the maintenance of extreme levels of polymorphism at these loci.

SO-10/P-40: Mapping of QTL modifying *Hbb-bh1* expression in *Klf1^{Nan}* F2 and Diversity Outbred (DO) mice

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Sickle cell disease (SCD) and β-thalassemia are hemoglobinopathies with high morbidity, mortality and biomedical costs. Elevation of fetal hemoglobin (HbF) by co-inheritance of positive genetic modifiers or hydroxyurea (HU) treatment ameliorates the diseases symptoms. However, HU has significant potential side effects that make the discovery of novel therapies elevating postnatal HbF urgently needed. Three major genomic loci are known to modify HbF expression. Together, they account for ~50% of the variation in HbF expression, indicating that additional modifiers exist. The overall goal of this study is to identify novel genetic regulators of β-like globin switching. The first approach is to map quantitative trait loci (QTL) that modify embryonic *Hbb-hb1* (βh1) globin expression in an F2 mouse model of *Klf1^{Nan}* neonatal anemia. *Klf1^{Nan}* is a missense mutation (E339D) in the second zinc finger of Krüppel-like factor 1 (erythroid) (*Klf1*) causing severe anemia accompanied by a failure of hemoglobin switching in heterozygous *Klf1^{Nan}*/+ mice (homozygotes die *in utero*). βh1 globin expression is upregulated in *Klf1^{Nan}* adult spleen and E14.5 fetal liver. Meanwhile, expression of *Bcl11a*, a downstream target of KLF1 and a major repressor of fetal globin in humans and mice, is expressed at haplosufficient levels, indicating that upregulated βh1 expression in *Klf1^{Nan}* is BCL11A independent. We produced an F2 intercross between C57BL/6J and 129S1.Cg-*Klf1^{Nan}*/ Llp and are also mapping modifiers of βh1 expression in Diversity Outbred (DO) mice, a high resolution mapping population. Preliminary statistical analysis on 173 (C57BL/6J x 129S1.Cg-*Klf1^{Nan}*/Llp)F2 mice and 261 DO mice identify QTL overlapping three loci known to influence β-like globin gene switching, providing proof of principle for our strategy. More importantly, additional loci identified contain no known modifiers, indicating the influence of novel genes.

SO-11/P-19: The genetic basis of morphological variation in the skull of the gray short-tailed opossum, *Monodelphis domestica*.

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Although there have been many studies attempting to identify genomic regions affecting variation in craniofacial morphology in mammals, the vast majority of these studies has focused on eutherians. As we move to a more genomic era of studying complex phenotypes, genetic mapping is becoming increasingly comparative. Marsupials have already become a valuable model organism for several human conditions, such as skin and eye cancer, hypercholesterolemia and spinal cord injury. This research aimed to expand the use of marsupial models to craniofacial research. The interest in marsupials is based on their overall levels of integration among craniofacial elements, since they are much higher than in eutherians. This high craniofacial integration in marsupials suggests that mammals underwent broad changes in the genetic underpinnings of craniofacial variation throughout their evolutionary history. Therefore, in this research project, we took advantage of a captive F2 intercross population of the gray short-tailed opossum (*Monodelphis domestica*) and carried out a genetic analysis of cranial morphological variation in this species. In this analysis, novel DNA surveying techniques (GBS) were used to generate molecular markers whose variation was correlated to individual differences in cranial morphology, measured through the use of 3D digitizing equipment. Several quantitative trait loci (QTL) contributing to individual differences in craniofacial morphology were then identified using statistical methods tailored to complex pedigrees. Our results suggest that, on average, marsupial QTLs have a wider range of pleiotropic effects than QTLs identified in studies of eutherians mammals. Pleiotropy is, therefore, the genetic basis for the high craniofacial integration in marsupials. In the future, we expect to identify interesting candidate genes within our QTL regions that will open up new research avenues for this model species.

SO-12/P-75: Quantifying allele-specific alternative splicing in personalized genomes using EM algorithm

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Current RNA-seq analyses employ three separate pipelines to quantify gene expression abundance, Allele-specific gene expression, and alternative splicing from RNA-seq data. Gene-level abundance is estimated from alignment of all reads genome-wide, ASE is assessed by analyzing only reads that overlap known SNP locations; and alternative splicing is estimated by analyzing reads overlapping splice junction or exon-specific reads.

Earlier, we developed computational tools; Seqnature and EMASE, to build individualized diploid genome using strain-specific variations or phased human genetic variations, perform personalized RNA-seq analysis using diploid transcriptome, and estimate allele-specific expression and gene expression simultaneously.

Here we extend EMASE with splice-aware EM algorithm to quantify allele-specific alternative splicing in addition to allele-specific expression and total gene expression simultaneously. The EM algorithm probabilistically allocates both allelic and genic multi-mapping reads to estimate effective read counts at exons and splice-junctions. These effective exon and splice-junction read counts can be readily used to get allele/isoform/gene expression estimates. We demonstrate the utility of the approach by using simulated data, real RNA-seq data, and single cell RNA-seq data from B6xCAST F1 hybrid mice.

SO-13/P-78: High-density lipoproteins mediate microRNA intercellular communication in type 2 diabetes.

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microRNAs (miRNAs) have proven to be powerful regulators of glucose metabolism and contribute to the pathogenesis of type 2 diabetes (T2D). Recently, we reported that high-density lipoproteins (HDL) transport and deliver functional miRNAs to recipient cells. Here we present that HDL-mediated intercellular communication is suppressed in T2D and restored with diabetic drug treatments. Using high-throughput small RNA sequencing, we found that 49 HDL-miRNAs were significantly reduced in Zucker Diabetic Fatty (ZDF) rats, a model of T2D, compared to lean controls. Colesevelam, a bile acid sequestrant that improves beta cell function, was found to correct (increase) 49% (24/49) of the HDL-miRNA changes. *Mir375,* a key regulatory miRNA in pancreatic beta cells, was found to be exported from beta cells to HDL, and this process was severely impaired (-4.5 fold) by hyperglycemic conditions *in vitro*. Using Trans-PAR-CLIPseq, a method that allows for the tracing of miRNAs between cells using a modified ribonucleoside, we found that beta cell-originating miRNAs, namely *Mir375*, was transferred to recipient endothelial cells and Huh7 hepatocytes, and loaded onto cellular argonaute RISC catalytic subunits 2 and 3 (AGO2/3)-RNA-induced silencing complexes. These data suggest that HDL-transferred *Mir375* was functional in recipient cells. Additionally, we found that HDL-*Mir375* expression in humans was similarly reduced in T2D, yet corrected with Colesevelam treatment after 8 weeks compared to placebo. Collectively, our data suggest that miRNAs in plasma, livers, endothelial cells, and pancreatic islets are highly sensitive to glycemic conditions and T2D, and that HDL-miRNA transfer may represent a novel form of endocrine-like communication in T2D.

SO-14/P-82: Evaluating the ENU landscape and its impact on ageing phenotypes.

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The Harwell Ageing Screen is a phenotype-driven screen, which mutagenizes male mice (G0) with the chemical N-ethyl-N-nitrosourea (ENU) to induce random single nucleotide variations (SNV) in the spermatogonia. Subsequent generations (G3) of mutant mice are then subjected to an array of phenotype procedures and monitored for phenodeviants over an 18-month period. To facilitate rapid gene identification the G1 genome was sequenced (WGS) to identify all potential causative loci.

Currently our dataset consists of 24 G1 WGS providing a platform to study the ENU landscape. We found the ENU-induced genome contains approximately 45 coding variants, where the vast majority are missense (85%). Furthermore, using ENCODE we found approximately 198 SNVs are located in regulatory regions of the genome, with the greatest number found in promoter regions (58%) and lincRNAs (23%). However, novel small insertions, deletions and larger SVs were rare and only seen in a few genomes. We examined the randomness of the ENU distribution by designing a Monte Carlo simulation of the SNVs to determine if there were any hotspots. We found a very small proportion of the genome (<80Mb) contained a significant overrepresentation of SNVs. Lastly we have mapped several mutants and potential causative variants have been confirmed via traditional Sanger sequencing resulting in a 96% success rate in SNV validation.

All predicted SNVs alongside numerous significant ageing phenotypes are disseminated via MouseBook (www.mousebook.org). The database currently holds 214 pedigrees with phenotypes annotated to 86 parameters in 15 procedures over an 18-month period. The SNV section currently holds approx. 15000 variants, which can be queried via their functional annotations such as miss-sense and stop-gained. All relevant gene-containing SNVs are associated to known rare human disease in Orphanet. This is a unique resource that shows the pleiotropic effects of ageing alongside all the predicted variants in the genome.

SO-15/P-48: Mapping host-pathogen genetic interactions to understand TB pathogenesis

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The complex interplay between host and bacterial pathways during *Mycobacterium tuberculosis* (*Mtb*) infection is poorly understood. To explore the genetic interactions between host and pathogen, we used a systems genetics approach that exploited both comprehensive bacterial genetics and diverse panels of recombinant inbred mice. One panel was derived from a C57BL/6 x DBA/2 cross (BXD) and a second more diverse panel was comprised of an 8-way cross of classical lab and wild-derived mice (Collaborative Cross, CC). We infected both panels with saturated libraries of *Mtb* transposon mutants to examine interactions between mouse genotype, the extent of disease, and the requirements for specific bacterial virulence functions. We quantified a wide variety of disease phenotypes, including bacterial burden, replication dynamics, wasting, and histopathology. We found that the disease spectrum of the CC lines exceeded that of both the parental strains and previously characterized inbred lines. Host disease metrics were correlated with the differential fitness of *Mtb* mutants through transposon sequencing (Tnseq) analysis of *Mtb* libraries recovered from each mouse line. Quantitative trait loci (QTL) analysis is underway to identify mouse polymorphisms that are associated with specific alterations in disease and bacterial mutant fitness traits. The combination of systems genetics approaches undertaken in the current study will allow us to map genetic interactions between the host and pathogen that determine disease outcome, and generate novel small animal models of TB that better replicate human disease states.

SO-16/ P-50: Spontaneous 8bp deletion in *Nbeal2* recapitulates the grey platelet syndrome in mice

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During the analysis of a whole genome N-Ethyl-N-Nitrosourea (ENU) mutagenesis screen for thrombosis modifiers, an 8 bp deletion causing a frameshift in exon 27 of the *Nbeal2* gene was identified. Analysis of the associated mouse pedigree demonstrated absence of this deletion allele in the original ENU mutagenized progeny and further genotyping identified a cohort of 129S1/SvImJ mice purchased from Jackson Laboratory as the likely source of the deletion variant. Though identification of a novel *Nbeal2* mutation in this ENU screen initially appeared as a plausible thrombosis modifier, in subsequent genetic analysis, this mutation failed to suppress the synthetic lethal thrombosis on which the genetic screen is based. Taken together with the demonstration that this mutation was not ENU-induced, and actually arose in the JAX mouse colony, these results serve as an instructive cautionary tale.

Mutations in *NBEAL2* were recently shown to be the cause of the autosomal recessive form of Gray Platelet Syndrome (GPS), a rare bleeding disorder characterized by macrothrombocytopenia and gray appearing platelets due to lack of platelet alpha granules. Mice homozygous for the 8 bp frameshift in *Nbeal2* ($Nbeal2^{gps}/Nbeal2^{gps}$) exhibit a phenotype reminiscent of human GPS. The platelet count was significantly reduced in $Nbeal2^{gps}/Nbeal2^{gps}$ mice compared to wildtype controls ($p = 1.63 \times 10^{-7}$). In addition, $Nbeal2^{gps}/Nbeal2^{gps}$ platelets appeared pale on Wright-Giemsa staining and alpha granules were markedly reduced in platelet sections assessed by transmission electron microscope. These findings are consistent with previously published reports of mice with targeted disruption of *Nbeal2*. Though not noted in these previous reports, we also observed a significant decrease in the number neutrophils in $Nbeal2^{gps}/Nbeal2^{gps}$ mice ($0.27 \pm 0.19 \times 10^3$ cells/μl compared to $0.77 \pm 0.32 \times 10^3$ cells/μl in controls, $p=2.44 \times 10^{-9}$). In contrast to previous reports, we did not observe a significant increase in mean platelet volume.

Oral Presentations

O-01: Genetic Architecture of Meiotic Drive: Mapping Genes that Distort Random Chromosome Segregation in Female Meiosis

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We have recently described a new meiotic drive system on mouse Chromosome 2. The responder locus, known as *R2d2,* is located 83 Mb from the centromere and the allele subject to preferential transmission is a copy number expansion of a 127 kb cassette that spans the *Cwc22* gene. The expansion is present in multiple classical and wild-derived inbred strains, in commonly used outbred stocks and in wild mice. This system is thought to explain maternal transmission ratio distortion (TRD) in the Collaborative Cross (CC), the Diversity Outbred (DO), two interspecific backcrosses, a standard F2 intercross and an advanced intercross line. The presence and level of TRD at *R2d2* in the progeny of F1 female hybrids heterozygous for *R2d2* depends on unlinked modifiers (i.e., distorter genes). To identify the loci responsible we generated 211 females derived from the CC and DO that are heterozygous for the *R2d2* copy number expansion. Each female was mated to an FVB/NJ male and over 5,500 progeny were genotyped at *R2d2*. In addition, we performed a second mapping experiment using TR in the progeny in over 50 nuclear families in which the dam is heterozygous for the *R2d2* copy number expansion. These families were derived from two different generations of HR8xC57BL/6J advanced intercross line. Mapping was performed independently in both experiments using the MegaMUGA genotypes of heterozygous dams. We will discuss the biological, evolutionary and technological implications of our results.

O-02: PRDM9 drives the evolution of recombination hotspots

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Meiotic recombination generates new genetic variation and assures the proper segregation of chromosomes in gametes. PRDM9, a histone methyltransferase, initiates meiotic recombination in most mammals by using its highly polymorphic zinc-finger array to bind DNA at sites termed hotspots, where it trimethylates histone H3K4, creating the site where DNA double-strand breaks will occur. The mechanisms of DSB repair suggests that hotspots should exhibit self-destructive behavior over evolutionary time, yet genome-wide recombination levels remain relatively constant, a conundrum known as the hotspot paradox. To test if PRDM9 drives this evolutionary hotspot erosion, we compared activity of the *Prdm9*^{wm7} *(Prdm9*^{Cst}*)* allele in two *Mus musculus* subspecies backgrounds, *M.m. castaneus*, the subspecies in which *Prdm9*^{Cst} arose, and *M.m. domesticus*, which diverged approximately 750 thousand years ago, and into which *Prdm9*^{Cst} was introduced experimentally. Comparing these two strains, we find that genetic differences at PRDM9 binding sites lead to qualitative and quantitative changes in PRDM9 binding and activity. Using *Mus spretus* as an outlier, we found that most mutations affecting PRDM9 binding arose and were fixed in *M.m castaneus* and suppress hotspot activity. Moreover, *M.m castaneus* x *M.m domesticus* F1 hybrids exhibit a novel class of hotspots with large haplotype biases in both PRDM9 binding and chromatin modification that are absent from both parents. Analyses showed that these novel hotspots represent sites of historic hotspot loss that now become activated in F1 hybrids due to crosstalk between one parent's *Prdm9* allele and the opposite parent's chromosome. Together these data support a model where haplotype-specific PRDM9 binding in heterozygotes directs biased gene conversion at nearly half of all hotspots, ultimately leading to hotspot erosion.

O-03: Estrous cycle: a transcriptomic analysis of 4 regions of the murine brain

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For many years biomedical research, and in particular neuroscience research, has often focused on male subjects. Female subjects have frequently been excluded due to the unknown effects of the hormonal changes across the estrous cycle and the perceived complications of including the appropriate control groups. We utilized transcriptomic analysis in the hypothalamus, hippocampus, neocortex, and cerebellum of female C57BL/6J (B6) mice to examine the changes in gene expression in each of the four stages of the mouse estrous cycle. There are changes in gene expression across all stages of the estrous cycle. A few changes that are noteworthy include genes involved in circadian rhythm in the neocortex (*Per1*, *Per2*) that have decreased expression during estrus. Also, as might be expected, the expression of hormonal genes in the hypothalamus (e.g. *Prl, Gh*) varies greatly across the stages. Expression similarity network analysis is being used to explore: 1) the differences in transcript levels, and 2) the differential control of transcript abundance across the estrous cycle. We expect that our results will be a useful guide for researchers in the field of neuroscience.

O-04: Integration of Mouse ENCODE Data with mRNA Expression QTL Data

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The ability to discover and model transcriptional regulation would greatly enhance our ability to predict cellular responses to external stimuli. As a first step toward this goal, we sought to discover candidate genomic variants that may explain transcriptional variation in a genetically diverse population of mice. We measured transcript levels in the livers of 469 Diversity Outbred (DO) mice of both sexes and estimated gene expression. We genotyped these mice using the Mouse Universal Genotyping Array, imputed single nucleotide polymorphisms (SNPs) from the founder genomes onto the DO genomes and performed association mapping in a 10 Mb window around each transcript's genomic location. We selected SNPs that were significantly associated with transcript expression and intersected these with regulatory elements from The Mouse Encyclopedia of DNA Elements (ENCODE). We searched for transcription factor binding sites in DNase hypersensitive and epigenetic regions that intersect with the most significant SNPs and present ways of visualizing these relationships. This analysis provides a set of candidate regulatory SNPs that can be further interrogated through *in vitro* or *in vivo* methods to build a predictive model of liver gene expression in DO mice.

O-05: A new look at the genetic basis of F1 hybrid sterility of mouse inter-subspecific hybrids

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The F1 hybrids between *Mus m. musculus* (PWD/Ph) and *Mus m. domesticus* (C57BL/6) show extensive failure of meiotic chromosome synapsis and subsequent male sterility. The extent of asynapsis and meiotic arrest are controlled *in trans* by the Dobzhansky_Muller incompatibility between *Prdm9* and *Hstx2*. This interaction is necessary but not sufficient, as shown by a reconstitution experiment in (C57BL/6J-Chr X.1s ^{PWD}/ForeJ × C57BL/6J-Chr 17 ^{PWD}/ForeJ) chromosome substitution strain hybrids, which carried the same allelic setup of both genes on C57BL/6 background but did not provoke asynapsis or sterility. To inquire into the mechanism of meiotic asynapsis, a series of sterile (PWD x C57BL/6J-Chr # ^{PWD}/ForeJ) F1 hybrids were analyzed, in which both homologs of a particular autosomal pair came from the same parental strain (PWD). Combining immunofluorescence microscopy of SYCP3 and HORMAD2 proteins and whole chromosome DNA FISH we found that asynapsis was characteristic for heterospecific chromosomes only. Moreover, synapsis of heterospecific (PWD/B6) chromosomes can be rescued by ≅ 20 Mb of a conspecific (PWD/PWD) sequence anywhere along the chromosome pair. Based on these findings we propose the *cis*-controlled asynapsis of chromosomes from two different (sub)species as a primary cause of apoptosis of meiotic cells and male sterility. The male-specific action of *Prdm9* and *Hstx2* can explain why the same perturbation of orthologous sequences acts differently in male and female meiosis.

O-06: Genetic regulatory variation between inbred mouse strains

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Our goal is to understand the biological mechanisms of how genetic variants result in complex trait variation. Gene expression differences between individuals explain a substantial portion of phenotypic differences between those individuals. Therefore, variants in gene regulatory elements are potentially very important in the context of complex traits. We identified open chromatin regions in embryonic fibroblasts (MEFs) from eight inbred mouse strains using DNase I hypersensitivity. Inbred strain MEFs showed between 126,168 and 174,754 regulatory regions that comprised 0.35% of the genome. Of these, 88778 regions varied between inbred strains. The numbers of differentially open chromatin regions between strains was proportional to the phylogenetic distance between those strains, which suggested that the chromatin landscape is controlled by many regulatory variants that accumulated gradually over the evolutionary history of *Mus musculus.* We related variable regions to local sequence variation and gene expression levels at nearby genes. These results demonstrate the utility of MEFs as a tool to unravel the genetic basis of global gene regulation.

O-07: *Zic2* mutation causes holoprosencephaly via disruption of NODAL signalling

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Holoprosencephaly (HPE) is the most common developmental defect of the forebrain with a prevalence as high as 1:250 during human embryogenesis. It is a major cause of both intra-uterine pregnancy loss and post-natal mortality and morbidity in affected cases. HPE occurs when the forebrain fails to separate into distinct left and right halves. The aetiology of HPE includes a teratogenic and genetic basis with both single gene defects and chromosomal imbalances associated with HPE. Mutations of the transcription factor *ZIC2* account for at least 8% of detectable HPE and it is one of the genes most frequently associated with HPE. Analysis of *ZIC2* mutations suggests loss-of-function as the likely pathogenic mechanism. Consistent with this the kumba ($Zic2^{Ku}$) mouse model of *Zic2* loss-of-function exhibits HPE. The genes associated with human HPE can usually be assigned to one of two signal transduction pathways, either the NODAL or SHH pathway. During gastrulation the NODAL pathway generates a signaling centre beneath the developing forebrain. Slightly later, at the early organogenesis stage, this signaling centre secretes SHH which patterns the overlying neural tissue. We have used mouse genetics to demonstrate that ZIC2 does not act within the SHH pathway to cause HPE, instead, it is required during gastrulation to produce the signaling centre that will later secrete SHH. The establishment of this centre is under the control of NODAL signaling. Recently we have shown that *Zic2* mutation causes defects in cardiac situs, another NODAL regulated process. Now we have used mouse genetics to show that ZIC2 promotes NODAL signaling during gastrulation. We have also used biochemical analysis to uncover the molecular nature of ZIC activity downstream of NODAL signaling. ZIC2 fine tunes the transcriptional response to NODAL signaling, identifying for the first time the molecular basis of ZIC2-associated HPE.

O-08: A role for adenosine monophosphate deaminase in malaria resistance

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Malarial parasite resistance to all known antimalarial drugs is now the norm. Parasites develop resistance through modification of both target and intra-parasitic drug concentrations. We have developed a strategy to develop new therapies that will bypass both these mechanisms of resistance. ENU mutagenesis is used to introduce mutations into the germline of mice that are otherwise susceptible to murine malaria. Mice carrying protective mutations will survive a malarial challenge whereas all other mice will succumb. The genes harbouring the mutations are identified and assessed as potential antimalarial drug targets. At present we have identified 40 mutations conferring resistance to malarial infection and have 80 resistant lines. Here, we propose a novel mechanism of host resistance to malaria infection. *Ampd3^{MRI49372}* carries a dominant mutation in the erythrocyte specific isoform of AMP deaminase. This enzyme regulates a step in purine metabolism crucial for maintaining the balance between ATP and GTP within the red blood cells. The mutation – T689A – causes a gain of function in the enzyme, resulting significantly elevated GTP levels in homozygotes. Mutants also exhibited marked increase erythropoiesis. This is likely due to dramatically increased red cell turnover. When challenged with the rodent malaria parasite, *P. chabaudi*, *Ampd3^{MRI49372}* proved extremely resistant, with both homozygous and heterozygous mutants showing a dramatic increase in survival. We propose that the malaria resistance of *Ampd3^{MRI49372}* line is mediated by the short RBC half-life and the antimalarial effect of GTP. This study provides the first evidence that a mutation in *Ampd3* can lead to malaria resistance in mice, and shows that balanced host purine metabolism is essential for supporting the growth of *Plasmodium* spp within Red Blood cells.

O-09: A missense, gain-of-function mutation in *Tmem98* causes Bochdalek congenital diaphragmatic hernia

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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 are no non-syndromic mouse models for this condition. Mapping placed *Rwhs* in a small interval on Chromosome 11, sequencing of which uncovered one nucleotide substitution which changes a highly-conserved isoleucine to threonine (I135T) in *Tmem98*, a novel gene predicted to encode a transmembrane protein. Two different loss-of-function alleles of *Tmem98* (*Tmem98$^{tm1a(EUCOMM)Wtsi}$* and *Tmem98^{m1H}*) are homozygous lethal because of mid-line body wall closure defects. The diaphragm is unaffected. Both complement the I135T allele, i.e. compound heterozygotes are viable. BAC transgenic experiments indicate that I135T is a gain-of-function mutation that acts in a recessive manner, most likely because a threshold level of the mutant protein is required to cause the mutant phenotype. Up to this threshold level the I135T protein does not have a detrimental effect and can compensate for loss-of-function. In line with this, modelling the effect of the I135T mutation on TMEM98 protein structure indicates that it is only very mildly destabilising. Retinoic acid signalling is known to be important for diaphragm development and we are currently investigating if this pathway is perturbed by the I135T mutant TMEM98. In summary, loss of *Tmem98* function leads to body wall closure defects whereas a threshold level of the I135T allele causes diaphragmatic hernia. This very interesting diaphragmatic hernia phenotype is specific to the I135T mutation and would be unlikely to be discovered by any other method than ENU mutagenesis.

O-10: Consequences of the Loss of Cholesterol Synthesis in the CNS by Inactivation of the Cholesterologenic Enzyme NSDHL are Developmental-Stage and Cell Type Specific

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NSDHL is a 3β-sterol dehydrogenase involved in the removal of C-4 methyl groups in post-squalene cholesterol biosynthesis. Mutations in the gene are associated with the X-linked, male-lethal mouse mutation bare patches, *Bpa*; hypomorphic human mutations are a rare cause of male intellectual disability. Since male *Nsdhl* mutant mouse embryos die by midgestation, we generated a conditional targeted allele (*Nsdhl$^{tm1.1Hrm}$;* called *Nsdhl^{flx5}*) to study mechanisms for later developmental malformations and postnatal effects associated with NSDHL deficiency. Global inactivation of the gene in the early embryo (*Nsdhl$^{tm1.2Hrm}$*, called *Nsdhl$^{\Delta 5}$*) replicates the homozygous *Bpa* phenotype. We next examined phenotypes at different stages and in different cell types of the CNS using a series of cre recombinase expressing lines. Inactivation of *Nsdhl* using B6.Cg-Tg(Nes-cre)1Kln/J results in perinatal lethality while affected males from matings with FVB-Tg(GFAP-cre)25Mes/J develop severe wasting and ataxia at ~P7 and die between P10-P20. They demonstrate cerebellar granule cell proliferation and migration defects and a markedly reduced response to SHH *in vivo* and *in vitro* in cultured cerebellar granule cells. The *in vitro* defects can be rescued by treatment with exogenous cholesterol. Inactivation in cortical neurons of the adult CNS (starting ~P19) using B6.Cg-Tg(Camk2a-cre)T29-1Stl/J produces no observable phenotype, including performance in a series of behavioral assays. In contrast preliminary data show that *Nsdhl$^{\Delta 5}$* males from STOCK-Tg(Fgfr3-icre/ERT2)4-2Wdr matings (cre induction at P45) showed a significant increase in self-grooming relative to controls. Mutant males spent less time in the open in the elevated-plus maze and were less aggressive in the tube test for social dominance. These data demonstrate an essential role for cholesterol in the postnatal CNS with obligatory sites of synthesis changing from neurons to astrocytes as pups mature. Further studies will examine effects in older mice as well as possible mechanisms for the features noted above.

O-11: Postsymptomatic rescue of clinically relevant phenotypes in a new mouse model of Spinal Muscular Atrophy

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Mouse models of spinal muscular atrophy continue to provide valuable insight into disease pathology and therapeutic efficacy. Modeling human conditions where disease severity is linked to insufficient or low levels of an essential protein poses a great challenge as often disease phenotypes and severity present in mouse models differ from that seen in humans. Here we present an intermediate mouse model of SMA with both disease related and comorbidity phenotypes. We investigate the ability of systemic administration of *SMN* inducing therapeutics to correct both SMA and non-SMA like phenotypes in this mouse model at different stages of disease. We generated an intermediate model of SMA by combining a transgene encoding the entire human genomic *SMN2* promoter and gene along with two targeted mutations of the murine *Smn1* locus: one bearing two tandem hybrid (mouse/human)*Smn1/SMN* genes and one encoding a disrupted exon 2 abolishing gene expression. These mutants displayed phenotypes associated with SMA including reduced expression of SMN, shortened lifespan, and neuromuscular junction defects (NMJs). NMJs of transverse abdominus (TVA) muscle in these mutants exhibited a maturational delay characterized by a shift in the ratio between immature "plaque" like NMJs and morphologically mature "pretzel" like NMJs. Mutants also exhibited comorbidity phenotypes including cardiac abnormalities characterized by decreased ejection fraction, fractional shortening, reduced heart rate along with increased intraventricular septum depth by 90 days of age. Similar to other mild mouse models of SMA, these mutant mice exhibited peripheral necrosis. Systemic administration of *SMN* inducing compounds at PND10 and PND25 resulted in the correction of both SMA-like and comorbidity phenotypes in this model.

O-12: Using human GWAS data to dissect metabolic traits in the Diversity Outbred mouse population

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Identification of genetic variants that contribute to complex metabolic traits has had limited success in conventional mouse strain crosses due to low-resolution mapping. The Diversity Outbred (DO) population addresses this issue by mimicking the genetic diversity of the human population but introduces new challenges in that large numbers are needed to test all possible genetic differences similar to the large cohorts needed for human GWAS studies. We developed an analysis pipeline that controls the false discovery rate by only testing for genes previously implicated in metabolic disorders. 849 DO mice were fed high fat or chow diet and were phenotyped for over 100 metabolic traits. Mice were divided into two groups, determined by percent body fat as being obese (OB) or non-Obese (non-OB) and genotypes were determined for 671 genes orthologous to human genes associated with metabolic syndromes in the Human GWAS Catalog. An additive genotype associative test was performed per trait and the SNP with the lowest p-value per gene was selected for further analysis. In the OB group, 1483 gene*trait pairs have significant differences between alleles for a measured trait. These include biologically interesting findings such as the locus around *Fitm2* where the CAST/EiJ founder allele contributes to increased area under the curve in the glucose tolerance test, and the locus surrounding *Rasal2* where the 129S1/SvImJ founder allele contributes to higher circulating non-esterified fatty acids. Likewise, 596 gene*trait pairs were found to be significant in the non-oB group, including a locus containing *Pparg* in which the A/J allele drives high circulating levels of bilirubin, a trait known to be negatively correlated with obesity in humans. Our results demonstrate that analysis of metabolic traits in DO mice is enhanced by integrating findings from human GWAS studies.

O-13: Comparison of Tumorigenesis in Mouse Models of Metastatic Colorectal Cancer

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Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the US, with mortality almost exclusively due to metastatic disease. Therefore, understanding the mechanisms governing how an adenomatous polyp develops into metastatic carcinoma is of great clinical importance. There is increasing evidence suggesting that metastatic potential is a heritable trait, rendering some individuals at greater risk for developing metastatic disease. The goal of this study is to develop a mouse model of CRC that will allow the investigation of whether genetic background influences tumor progression and metastases through interaction with clinically-relevant mutations.

We compare mouse models of CRC initiated by different mutations in the WNT signaling pathway, including a homozygous, conditional knock-out allele of adenomatous polyposis coli allele (Apc^{tm2Rak} or Apc^{f}) and a heterozygous, conditionally-stabilized beta-catenin allele ($Ctnnb1^{tm1Mmt}$ or $Ctnnb1^{f(Ex3)}$). A dominant-acting murine model of tumor initiation such as $Ctnnb1^{f(Ex3)}$ will be valuable in investigating genetic influences on CRC metastasis susceptibility. CRC progression is influenced by the presence of specific cooperating mutations, which can also be incorporated into our murine model. The conditional alleles are activated by a cre recombinase-expressing adenovirus (AdCre) that is delivered to the distal colonic lumen. Tumor growth is assessed by serial colonoscopy.

While we found that the $Ctnnb1^{f(Ex3)}$ allele alone is generally not sufficient to induce tumorigenesis following AdCre delivery, the presence of additional cooperating mutation(s) is highly tumorigenic. Transcriptome analysis and histology reveal differences between $Ctnnb1^{f(Ex3)}$ and Apc^{f}/Apc^{f} initiating events in conjunction with the various conditional cooperating mutations. Based on the high penetrance of primary tumor formation in $Ctnnb1^{f(Ex3)}$/+ mice with cooperating mutations, and the observance of metastases, we are confident that we have developed a metastatic mouse model of CRC that exclusively utilizes dominant-acting alleles. This model will be valuable is investigating the influence of genetic background on CRC metastasis susceptibility.

O-14: A Critical Role for Cyclin D1 in Testicular Germ Cell Tumorigenesis

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In the 129 inbred mouse model of human testicular germ cell tumors (TGCTs), tumors initiate between embryonic days (E)13.5 to 15.5, which coincides with the mitotic:meiotic switch: germ cells of both sexes lose pluripotent capacity, female germ cells (oogonia) commit to meiosis, and male germ cells (gonocytes) enter mitotic arrest. Based on our previously published data that gonocyte proliferation, retention of pluripotency, and premature differentiation were directly related with increased tumor risk, we hypothesized that aberrant expression of *Ccnd1* significantly contributes to TGCT initiation by inducing a breakdown in the mitotic:meiotic switch, which causes gonocytes to continue proliferating and retain pluripotency. Using the $Ccnd1^{tm1Wbg}$ knockout on the high TGCT risk 129S1/SvImJ-Chr $19^{MOLF/Ei}$ (M19) background, we found that *Ccnd1* deficiency significantly reduced TGCT incidence by 62% ($p<0.001$) compared to wild-type and heterozygous animals. Immunohistochemistry experiments suggest that *Ccnd1* deficiency reduces the number of proliferating (KI67-positive) and pluripotent (NANOG-positive) gonocytes. Normally, *Ccnd1* expression in post-migratory embryonic germ cells is restricted to oogonia and is transiently expressed from E12.5 to E15.5, prior to their entry into meiosis. Expression of *Ccnd1* from E12.5 to E15.5 in TGCT-susceptible gonocytes suggests that a signal normally restricted to the developing ovary is aberrantly active in the TGCT-susceptible testis. Retinoic acid (RA) signaling normally precedes *Ccnd1* expression in embryonic oogonia, while RA is normally catabolized by CYP26B1 in the embryonic testis. Curiously, we find an altered expression pattern of embryonic germ cell sex-specific genes in TGCT-susceptible gonocytes. Therefore, we tested whether gonocyte expression of *Ccnd1* is induced by an ectopic RA signal. Culturing embryonic testes in medium containing RA induced *Ccnd1* expression in gonocytes. We currently hypothesize that TGCT-susceptible gonocytes respond to both an abnormal RA signal and normal gonocyte developmental signals, which disrupt gonocyte entry into mitotic arrest during the mitotic:meiotic switch and lead to TGCT initiation.

O-15: QTL mapping of Electrocardiographic Parameters and Cardiac Gene Expression in the BXD Recombinant Inbred Strains: Novel Loci and Candidate Genes

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Slowing of the cardiac electrical impulse, evidenced by prolongation of the P-wave duration, PR and QRS intervals on the electrocardiogram (ECG), is a common mediator of potentially lethal cardiac arrhythmias in a number of common and rare cardiac pathologies. Prolongation of the PR interval is also a strong predictor of atrial fibrillation, the most prevalent sustained cardiac arrhythmia in the general population. Despite the significant genetic component in the determination of these ECG indices, the underlying genes remain largely elusive.

We here aimed to identify novel genetic loci and the underlying causal genes for these ECG parameters by exploiting their variability among the BXD recombinant inbred lines. To this aim we acquired ECGs in 18 BXD lines in the absence and presence of the cardiac sodium channel blocker flecainide, from which we measured the ECG parameters and assessed the occurrence of cardiac arrhythmias. Furthermore, we obtained data on cardiac transcript abundance and heart rate from 43 BXD lines. Quantitative trait mapping identified 11 novel, nominally significant, QTLs for ECG parameters These included a locus for both post-flecainide P-wave duration and the PR-interval (Chr 19; CEL-19_43912943 - rs13483641; 420 kb; LOD-score 6.4) and an adjacent locus (rs3672117-rs3710829, 5.3 Mb, LOD 2.95) for arrhythmias. Analysis of expression QTLs (eQTLs) overlapping with these ECG QTLs identified putative candidate genes whose transcript abundance is mapping to these same loci in the heart.

In conclusion we here present two novel loci on mouse Chromosome 19 for cardiac atrial-ventricular conduction in the BXD panel of murine recombinant inbred lines. Analysis of further BXD lines will be aimed at further dissection of these loci.

O-16: Genetic and Pharmacologic Inhibition of MTOR delays Thymic Lymphoma Formation and Decreases CDK6 Levels

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The PI3K/AKT/mTOR pathway is hyperactivated in 50-75% of T- cell acute lymphoblastic leukemias (T-ALL). To model the pharmacologic inhibition of this pathway, mice with constitutively-active AKT in T-lymphocytes (Tg(Lck-Akt2*)#Test) were crossed with mice with genetically-reduced MTOR expression ($Mtor^{tm1Lgm}$), resulting in $Mtor^{tm1Lgm}$ Tg(Lck-Akt2*)#Test mice (KD). T-lymphocyte development was arrested at the DN3 stage in KD thymuses. $Mtor^{WT}$ Tg(Lck-Akt2*)#Test mice developed thymic lymphomas at an average of 14 weeks of age, while KD mice had delayed tumor development (at an average of 24 weeks of age). Delayed tumor development was recapitulated in KD mice treated with an MTOR inhibitor, RAD001. Gene expression profiling (GEP) of thymic lymphomas in WT and KD mice revealed differential expression in 98 genes. *Cdk6,* which is crucial for T-cell development and carcinogenesis, was downregulated 7-fold in tumors from KD mice. CDK6 protein levels were also reduced in KD thymic tumors and in tumor cells treated with the MTOR inhibitor, rapamycin. Tumor cells from WT tumors were more sensitive to CDK4/6 inhibitors than cells from KD tumors. When 11 human T-ALL cell lines were treated with a combination of a CDK4/6 inhibitor (PD-0332991) and rapamycin, the combination was synergistic in all cells lines and pRB was significantly decreased by the combination. Thus, reduced MTOR expression delayed thymic tumor formation induced by activated AKT, and CDK6 may be a critical component of this delay. As a result, targeting the MTOR pathway and CDK6 simultaneously is a promising combination for the treatment of T-ALL.

O-17: A reciprocal translocation disrupts *Auts2* gene expression in a novel mutant strain.

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AUTS2 is a recently discovered gene of unknown function that has been implicated in neurodevelopmental disorders, mood disorders, and drug use. *AUTS2* mutations have been discovered in over sixty humans with a combination of autism, epilepsy, and intellectual disability. The *AUTS2* locus has also been linked in genome-wide association studies to inheritance of epilepsy, bipolar disorder, major depression, alcohol consumption, and heroin addiction. This unique, conserved gene is thus implicated in a broad range of neuropsychiatric disorders with profound societal impact. Despite the strong association of *AUTS2* with neurological disease, little is known about its function or regulation. We have developed a unique genetic tool, the T(5G2;8A1)16Gso (16Gso) mouse mutation, to study developmental pathways underpinning the expression of *AUTS2*-related sydromes, as well as to better understand the function of AUTS2. 16Gso is a reciprocal translocation mutant that presents with seizures, craniofacial defects, and abnormal social and exploratory behavior. In this study, we have determined that the 16Gso mutation falls 60kB downstream of the *Auts2* gene, and that *Auts2* mRNA expression is reduced in the brains of adult mutants. We compared AUTS2 brain expression between 16Gso mutants and wild-type mice, and identified a reduction in the total number of Purkinje cells and a disruption in cell distribution and morphology in the cerebellums of 16Gso mutants, as well as absence of AUTS2-expressing cells in the dentate gyrus of the hippocampus. We also mapped AUTS2 expression in differentiating mouse neuroblastoma cells, where it appears to play a role during dendrite extension and elongation. Based on the effect of *AUTS2* mutations in humans and the expression pattern of AUTS2, we are testing the hypothesis that AUTS2 expression is necessary for normal neurodevelopment on a cellular and systemic level, and that *Auts2* dysregulation leads to the seizures, craniofacial and behavioral defects seen in 16Gso mice.

O-18: Practical management of laboratory research mice used to model human disease

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As part of continual efforts by The Jackson Laboratory to ensure the health and well-being of production and research mice while promoting cost-effective, state-of-the-art research, we have conducted extensive evaluations of housing conditions, with a particular focus on housing density. Most research institutions follow recommendations for housing density set out by *The Guide for the Care and Use of Laboratory Animals (Guide),* These standards, first set in 1963, were based on best professional judgment at the time because experimental data were insufficient. The *Guide* recognized the paucity of information available to support these guidelines and encouraged alternatives as long as they were data-driven and based on sound science. Towards the goal of updating housing standards that optimally balance animal welfare with cost-efficiency, many studies have since been reported evaluating mice at densities greater than recommended, finding that mice can be housed more densely than currently suggested. Forty-nine years later, in the eighth edition of the *Guide*, standards for housing density remain unchanged despite substantial improvements in mouse husbandry and caging and the emergence of new studies.

We used high-throughput, noninvasive screens to comprehensively evaluate growth, behavior and physiology of inbred and hybrid mouse strains towards defining measurable parameters of general well being and to assess how they are affected by increased housing density. This work aims to inform researchers using laboratory mice about optimal housing conditions to facilitate the generation of robust, reproducible and translatable experimental results as important mouse models of human disease are developed worldwide. Our studies demonstrate that mice can be housed at up to twice the density recommended by the *Guide* with no adverse effects on physiology and well being and further suggest that increased cage density may promote improved well being in some strains.

O-19: 9.6% of mouse gene knockouts show abnormal neuroanatomy: a resource to identify genes related to intellectual disability in human

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Although intellectual disability affects 1-3% of the population, it is one of the least understood health problems. It is estimated that genetic lesions account for half of the currently undiagnosed cases. Despite recent successes in identifying some of the mutations responsible, it has been suggested that up to 1,000 more genes remain to be uncovered.

The large number of intellectual disability syndromes is due to many causal pathophysiological mechanisms. The diversity of mechanisms results in an array of quantifiable neuroanatomical abnormalities. To identify genes related to intellectual disability, we are collaborating with the Sanger Mouse Genetics Project (MGP), allied to the International Mouse Phenotyping Consortium (IMPC), to systematically study the neuroanatomy of the MGP/IMPC knockout mouse strains using a standardized set of 78 brain parameters.

So far, we have assessed brain defects in 425 knockout mouse mutants (this number will double by the end of 2014). These preliminary data yielded success with the identification of 20 known intellectual disability genes including *Ap4e1*, *Cenpj*, *Chd7*, *Mcph1*, *Msmo1* and *Ube3b* demonstrating the pertinence of our approach. We also discovered 21 other genes including *Mta1*, *Cfap36*, *Caprin2* and *Dusp3*, which when disrupted caused modification of brain structures.

Our study is the largest screen of brain morphology from the MGP/IMPC. It shows that we can detect abnormalities in about 10% of knockout mouse mutants, and that these translate into human pathology. This offers a complementary resource to human genetic studies.

O-20: Verne Chapman Lecture: Automated forward genetics.

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Forward genetics was historically a blind process. Mutations were introduced at random, and an organized search for a single causative mutation was undertaken only after a phenotype was observed. This search was undertaken in the absence of information concerning non-causative mutations. Neither their number nor their identity was known. The development of massively parallel sequencing platforms capable of covering whole exomes in depth allowed the discovery of most mutations induced by ENU within pedigrees of interest. Nonetheless, only a single mutation was considered to be of interest. Genetic mapping became the principal bottleneck in finding causative mutations, and many more phenotypes were generally declared than found. To assign functions to genes far more efficiently, we have developed a fully automated system for genetic mapping. Our approach is to identify all ENU induced mutations that alter coding sense in G1 mice, and to genotype the mutated sites in all G3 mice, prior to screening for phenotype. Approximately 600 pre-genotyped G3 mice are produced weekly in 20 pedigrees containing 20-50 G3 animals. Each pedigree bears an average of 70 mutations. Hence, about 1,400 mutations are examined in depth for their ability to cause phenotype(s). Screening data from these animals, probing more than 30 independent aspects of immune function, are uploaded to a computer. This triggers a computational search for linkage using recessive, additive, and dominant models of inheritance. Instances of linkage are immediately accessible for analysis, and in many instances, the causative mutation can be determined instantly, irrespective of novelty. Both qualitative and quantitative phenotypes can be mapped, and the number of phenotypes emanating from a pedigree does not complicate the analysis. As multiple alleles of individual genes accumulate within the growing database of mutations and phenotypes, they are combined into "superpedigrees," which can give overwhelming evidence of causation, or alternatively, can exonerate loci suspected of participation in a particular biological process. Our system is also capable of detecting complex phenotypes, occasionally observable in pedigrees derived from ENU mutagenized mice. It permits precise estimates of genome saturation. At present writing, 33,974 allelic variants of 13,843 genes have been tested in screens applied to 19,358 mice from 559 pedigrees. The mutation sites have been queried 1,581,652 times in aggregate to detect phenotypic effects. Many mutations affecting genes with known immunological function have been implicated as causative of phenotype. Many more genes not previously known to be involved in immunity have been implicated as well.

O-21: Hair graying as a model to investigate the genetics of stem cell maintenance

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Adult stem cells respond uniquely to age; some disappear while others lose regenerative capacity. For melanocyte stem cells (McSCs) that exist within the hair follicle, they undergo aberrant differentiation and depletion with age and this causes hair graying. Why McSCs respond to age in this fashion remains unclear, and elucidation of this question will contribute to our growing understanding of how stem cell functionality changes with time.

Using loss-of-function ($Sox10^{tm7.1(Sox10)Weg}$/$Sox10^{tm7.1(Sox10)Weg}$ Tg(Tyr-cre/ERT2)13Bos) and gain-of-function (Tg(Dct-Sox10)CF1-10Pav) experiments we previously found that McSCs depend on the tight regulation of the transcription factor *Sox10* to exist. Too little *Sox10* and McSCs fail to persist. Too much *Sox10* causes McSCs to prematurely differentiate and produces hair graying. Hair graying is exacerbated by haploinsufficiency for another transcription factor, *Mitf* ($Mitf^{mi-vga9}$/+ Tg(Dct-Sox10)CF1-10Pav*)*, suggesting that loss of McSC maintenance is a genetically modifiable trait. In order to explore this possibility we developed a simple method to quantify differentiated McSCs by the fact that they produce ectopic pigment, and that severity of ectopic pigmentation of McSCs is predictive of future hair graying. Using Tg(Dct-Sox10)CF1-10Pav to predispose animals to hair graying, we discovered that strain variation has a significant impact on hair graying susceptibility— by assessing ectopic pigmentation in F1 hybrids generated from mating C57BL/6-Tg(Dct-Sox10)CF1-10Pav to inbred mice (C3H/HeJ, 129S6/SvEv Tac, FVB/N Tac, Balb/CJ, and DBA/1J) we show that DBA/1J and Balb/CJ lines are particularly resistant to McSC differentiation.

The phenotypic similarities between Tg(Dct-Sox10)CF1-10Pav and aged McSCs suggests that identifying the modifying genes involved in preserving McSC maintenance in the context of *Sox10* overexpression may also reveal genes relevant to aging. These observations coupled with our recent ability to acquire McSCs from both young and aged mice for genome-wide analysis further elevates McSCs and hair graying as an amenable model to identify novel genetic networks involved in stem cell maintenance with age.

O-22: Kinetochore associated protein 1 is essential for germ cell development and male fertility

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Jagged tail-like *(jgl)* is a spontaneous mutation that arose in the BALB/cByJ-$Clcn1^{adr-mto2J}$ colony at The Jackson Laboratory and was aptly named after its associated, recessive kinked tail phenotype, which was reminiscent of an older mutation (*jg*, jagged tail) that also arose at The Jackson Laboratory and was published in 1964. In addition to skeletal defects, both *jg* and *jgl* cause recessive, male infertility. As part of our efforts in the Mouse Mutant Resource, we performed a complementation test to confirm that *jgl* and *jg* are allelic and mapped the *jgl* mutation to a ~12.5 Mb region on Chromosome 5. Whole exome sequencing identified a novel, nonsense mutation in *Kntc1* (kinetochore associated 1*)*; a gene that has not previously been associated with a mouse phenotype but is a homolog of the *Drosophila* gene, rough deal *(rod),* which is required for proper segregation of chromosomes during mitosis. Analysis of *Kntc1* expression confirmed that this mutation results in differences in transcript availability across multiple tissues and developmental time points from mutant and wild type animals. To examine the underlying causes of infertility, we performed histology on mutant testes and found severely disrupted spermatogenesis and germ cell depletion. Interestingly, quantitative analysis of follicle distribution in mutant female ovaries revealed a 50% reduction in follicle number, however breeding data from mutant females did not provide evidence of premature ovarian failure. To determine the developmental timing of germ cell depletion we examined mutant testes and ovaries at birth and at mid-gestation. Germ cells were depleted at birth in both sexes; therefore, germ cell depletion in *jgl* mutant animals likely originates during gestation. Interestingly, the organization of the testes vasculature was disrupted / delayed at E12.5, supporting a role for *Kntc1* in the development of the testes vasculature during organogenesis.

O-23: A Systems Genetics Approach Identifies *CXCL14*, *ITGAX*, and *LPCAT2* as Novel Aggressive Prostate Cancer Susceptibility Genes

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Although prostate cancer typically runs an indolent course, a subset of men develop aggressive, fatal forms of this disease. We hypothesize that germline variation modulates susceptibility to aggressive prostate cancer. The goal of this work is to identify susceptibility genes using the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of neuroendocrine prostate cancer. Quantitative trait locus (QTL) mapping was performed in transgene-positive (TRAMPxNOD/ShiLtJ) F2 intercross males (n = 228), which facilitated identification of 11 loci associated with aggressive disease development. Microarray data derived from 126 (TRAMPxNOD/ShiLtJ) F2 primary tumors were used to prioritize candidate genes within QTLs, with candidate genes deemed as being high priority when possessing both high levels of expression-trait correlation and a proximal expression QTL. This process enabled the identification of 36 aggressive prostate tumorigenesis candidate genes. The role of these genes in aggressive forms of human prostate cancer was investigated using two concurrent approaches. First, logistic regression analysis in two human prostate gene expression datasets revealed that expression levels of six genes (*C19orf57*, *CXCL14*, *ITGAX*, *LPCAT2*, *RNASEH2A*, and *ZNF322*) were positively correlated with aggressive prostate cancer and two genes (*CCL19* and *HIST1H1A*) were protective for aggressive prostate cancer. Higher levels of expression of the six genes positively correlated with aggressive disease and were consistently associated with patient outcome in both human prostate cancer tumor gene expression datasets. Second, three of these six genes (*CXCL14*, *ITGAX*, and *LPCAT2*) harbored polymorphisms associated with aggressive disease development in a human GWAS cohort consisting of 1,172 prostate cancer patients. This study is the first example of using a systems genetics approach to successfully identify novel susceptibility genes for aggressive prostate cancer. Such approaches will facilitate the identification of novel germline factors driving aggressive disease susceptibility and allow for new insights into these deadly forms of prostate cancer.

O-24: Mitochondrial dysfunction and age-dependent neurodegeneration

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Mice lacking the E3 ubiquitin ligase mahogunin ring finger 1 (MGRN1) have a pleiotropic phenotype that includes progressive, widespread spongiform neurodegeneration. Although vacuoles are not apparent until 9-10 months of age, mitochondrial dysfunction is detected in the brains of 1 month-old mutants. Mitochondrial dysfunction is associated with most forms of neurodegeneration, and mutations in *PARK2 (PARKIN)*, which encodes an E3 ubiquitin ligase that mediates the autophagic degradation of damaged, depolarized mitochondria, can cause early onset Parkinson's disease. We investigated whether MGRN1 plays a direct role in regulating mitochondrial homeostasis. We show that MGRN1 is tightly associated with mitochondria and that loss of MGRN1 function disrupts mitochondrial function *in vivo* and *in vitro*. MGRN1 did not affect parkin-mediated mitophagy, but mice lacking both MGRN1 and parkin showed more severe mitochondrial dysfunction and more rapid progression of brain vacuolation. These data suggest that MGRN1-dependent ubiquitination plays an important role in mitochondrial homeostasis that, when disrupted, leads to spongiform neurodegeneration.

O-25: An ENU-induced mutation in whirlin with a novel phenotype

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Using ENU mutagenesis at MRC Harwell to screen for age-related recessive phenotypes we have uncovered both late-onset and early-onset phenotypes including a novel mutation within *Whrn*, implicated in Usher Syndrome Type II. Whirlin is a cytoskeletal scaffold protein involved with stereocilia elongation and development in hair cells and is crucial to the localization and stability of the USH2 proteins, USH2A (Usherin) and GPR98 (VLGR1). Two main isoforms exist: a long isoform contains two N-terminal PDZ-domains, a proline-rich domain and a C-terminal PDZ-domain; and, a short isoform containing only a proline-rich domain and the C-terminal PDZ-domain.

Mutations reported within *Whrn* have resulted in primary sensory cell defects in both humans and mice. A spontaneous deletion, the whirler mutation, ablates the short isoform and truncates the long isoform, resulting in mice with profound hearing loss and a vestibular defect (Mburu et al., 2003) that is associated with shortened inner hair cell (IHC) stereocilia and irregular outer hair cell (OHC) stereocilia bundles. Mice carrying a knockout of the long isoform have a partially deaf phenotype but no vestibular defect and a late onset retinal degeneration (Yang et al., 2010) with abnormal OHC stereocilia bundles, but normal IHC stereocilia.

A donor splice site variant identified in our *Whrn* mutant (*Whrn*^{m1H}), gives rise to a neurological dysfunction that is demonstrated by a characteristic head bob phenotype. Mutants are typically hyperactive in the open field arena and in wheel running activity. However no deafness or obvious vestibular defects are observed in these mice. SEM images of ears from affected and unaffected mice revealed OHC stereocilia bundle irregularities, while the structure of IHC stereocilia is normal.

Overall, our results identify a novel role for whirlin, in a mutant that does not have a deafness phenotype and, could provide further insights into the function of whirlin isoforms in IHCs and OHCs.

O-26: Insights into Transcriptional Regulation by SOX10 from Genome-wide Profiling of Melanocyte Cistromes

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SOX10 is critical for the specification and maintenance of neural crest-derived melanocytes. Recently it has been shown that SOX10 levels play major roles in melanoma and drug resistance. Yet, the genomic targets of SOX10 and the contribution of chromatin modification have not been comprehensively defined. Here we provide the genome-wide profile of the cistrome of SOX10 and its transcriptome using chromatin immunoprecipitation coupled with DNA sequencing (ChIP-Seq), and gene expression microarray studies in proliferating melanocytes. ChIP-Seq analysis reveals that SOX10 genomic binding sites exhibit strong evolutionary constraint, and predominantly localize to gene bodies and distal, non-coding regulatory DNA elements, including, previously annotated putative melanocyte enhancers. Unbiased *de novo* motif analysis shows that SOX10 binds to DNA sequences of greater complexity *in vivo* in addition to the canonical SOX10 binding motif ACAA(A/T)G. SOX10 putative targets derived from ChIP-Seq analysis include several previously characterized genes as well as new targets. Functional gene expression analysis of control and *Sox10*-heterozygous (B6.129-*Sox10*^{tm1Weg}) melanocytes indicates that SOX10 mediates both transcriptional activation and repression. SOX10-activated genes are linked to pathways critical for pigmentation, cell survival and proliferation (e.g. *Tyr, Irf4, Erbb3*) versus negatively regulated genes with associated roles in development (e.g. *Kit*) and epithelial-to-mesenchymal transition (e.g. TGF-beta/SMAD and PDGF signaling pathway genes). Comparison of SOX10 binding motif signatures reveals remarkably similar enrichments at both SOX10-activated and -repressed genes. Interestingly, SOX10-activated genes and -repressed genes display distinct features of chromatin modifications and motif enrichments for other transcription factors. This suggests that SOX10-regulated transcriptional outcome is not specified by motif classification alone but is influenced by chromatin landscape and collaborating transcription factors. Our study uncovers a previously unappreciated role of SOX10 as a negative regulator of a subset of its target genes and also provides new insights into the mechanisms of genome regulation by SOX10 in the melanocyte-lineage.

O-27: Modelling rare human neurodevelopmental disorders in mice on a large scale

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Windows of Hope (WOH) is a family pedigree-based genetics project aimed at understanding the causes of inherited conditions in US Anabaptist populations. Deciphering Developmental Disorders (DDD) is a parent/child trio-based exome sequencing consortium that catalogues the genetic architecture underpinning rare neuro-developmental disorders in UK children. We are collaborating with both these initiatives to model many new genetic disorders in mice, to establish a causal link between the candidate genes and neuro-developmental phenotypes and potentially inform treatment. Here we describe examples of phenotypic analyses of mouse models derived from both projects.

The most frequently mutated candidate gene detected by DDD to date is *ARID1B*, a component of the BAF chromatin remodelling complex. Heterozygous predicted loss-of-function mutations in this gene were found in patients with moderate to severe intellectual disability with or without dysmorphic features and restricted growth. We find that *Arid1b-/-* (C57BL/6N-Arid1b$^{tm1a(EUCOMM)Hmgu}$/C57BL/6N-Arid1b$^{tm1a(EUCOMM)Hmgu}$) mice die mid-gestation, but heterozygous loss results in a comparable behavioural and morphological phenotype. The mice have a long-term memory deficit and impaired growth in comparison with controls. As in humans, the restricted growth is not associated with a skeletal dysplasia.

WOH recently identified nine related macrocephalic patients with mild intellectual disability, all with homozygous mutations in the actin binding protein gene, *KPTN*. Similarly, *Kptn-/-* mice (C57BL/6N-*Kptn*$^{tm1a(EUCOMM)Wtsi}$/C57BL/6N-*Kptn*$^{tm1a(EUCOMM)Wtsi}$) have macrocephaly and display behavioural deficits in both social and novel object recognition consistent with a cognitive impairment. We observe no anxiety phenotype, but mutants display mild hyperactivity, increased body weight and abnormal clinical chemistry compared to controls. KPTN associates with the actin cytoskeleton in hippocampal neurons, but this is lost in the mutant protein suggesting the cognitive phenotype is due to a defect in neuromorphogenesis. Having established mouse models that phenocopy these patients, we aim to 'revert' mutant alleles to wildtype *in vivo* to determine whether the cognitive disability can be rescued.

O-28: Mouse models for idiosyncratic tolvaptan-induced liver injury are identified using a Collaborative Cross approach

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The arginine vasopressin receptor 2 antagonist tolvaptan is a promising candidate for the treatment of Autosomal Dominant Polycystic Kidney Disease (ADPKD). However, FDA approval has not yet been received in part due to reports of idiosyncratic drug-induced liver injury (DILI) associated with tolvaptan use in ADPKD patients. Because DILI cases are relatively rare and nonclinical testing of tolvaptan in traditional animal models did not indicate a risk for liver injury, it has been challenging to understand the DILI associated with tolvaptan. The objective of this study was to evaluate the liver response to tolvaptan in the genetically diverse mouse strains of the Collaborative Cross (CC) and identify strains sensitive to the liver injury. Eight (8) mice in each of 45 CC strains were treated with a single oral (gavage) dose of either tolvaptan (100 mg/kg) or vehicle (hydroxypropyl methylcellulose). Vehicle- and tolvaptan-treated animals within each strain were treated in pairs, and pairs within each strain were randomized over the course of the study to minimize batch effects. Serum alanine aminotransferase (ALT) or aspartate aminotransferase levels, but not total bilirubin levels were elevated in tolvaptan-treated animals relative to vehicle-treated controls in 3 of the 45 strains ($p<0.05$, Bonferroni post test). In these sensitive strains, the individual fold change in plasma levels of *Mir122* were correlated with the fold change in ALT (Pearson's $r=0.5834$; $p=0.0595$), suggesting that ALT levels are indicative of hepatocellular necrosis. Average plasma tolvaptan concentration at 2 h post dose was also highest in the 3 sensitive strains. Phenotypic findings in combination with toxicogenomics will be used to identify variation in specific genes and pathways associated with susceptibility to tolvaptan-induced liver injury. Importantly, risk factors identified in this study will be used to generate hypotheses for testing in DNA obtained from patients who have experienced the tolvaptan liver toxicity.

O-29: Systems Genetic studies of Atherosclerosis and the novel Plasma Metabolite trimethylamine N-oxide (TMAO)

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The common forms of atherosclerosis involve a large number of genetic and environmental factors. While recent human genome-wide association studies have identified numerous loci contributing to both coronary artery disease and its associated risk factors, these studies are limited by the inability to control environmental factors and examine detailed molecular signatures such as gene expression of relevant tissues. Metabolomics, the system-wide study of small molecule metabolites, provides particularly useful data that aids our understanding of how common genetic variation affects disease susceptibility. For example, recent studies have identified that specific choline metabolites are predictive of cardiovascular disease risk and that plasma levels of these metabolites are influenced by diet and by the gut microbial composition. In particular, circulating levels of the choline metabolites trimethylamine N-oxide (TMAO) and betaine are associated with increased atherosclerosis in humans and mice.

We have initiated a set of studies across genetically diverse mouse strains to understand basal and diet-induced changes in choline metabolism and the relationship with atherosclerosis susceptibility. Initial results thus far demonstrate that choline metabolites vary by mouse genetic background and are affected by diet, and that dietary challenges elicit gene x diet interactions that may involve the microbiome. We report here a study of natural variations contributing to atherosclerosis and its risk factors from studies of inbred strains comprising the Hybrid Mouse Diversity Panel (HMDP) and the Diversity Outbred Population (DO). In particular, we are interested in understanding genetic regulation and underlying mechanisms by which the novel plasma metabolite trimethylamine N-oxide affects atherosclerosis susceptibility.

O-30: MGI at 25: looking back – looking forward

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The mission of Mouse Genome Informatics (MGI, http://www.informatics.jax.org) as stated on the MGI web site is to provide integrated genetic, genomic, and biological data about the laboratory mouse to facilitate the study of human health and disease. During its first 25 years, this has translated to constant and dramatic growth and change, as the science that MGI supports, the computing tools MGI uses for its infrastructure, and the way MGI supplies data and tools to users have rapidly evolved.

In this talk, we track the history of MGI from a pre-web version distributed via hard-copy and floppy disks to today's internet where we do not tolerate a few second lag in getting an "answer"; and from a nascent molecular biology beginning (think RFLPs and single gene experiments) to whole genome sequencing and interaction networks.

With all of the remarkable changes in how and what data are stored and how users can access and analyze data, the key underlying principle of MGI has remained: to provide the highest-quality data possible and, through careful integration of diverse data sets, provide a platform for new hypotheses and discovery.

It has been an exciting ride and we can't wait to see how the next 25 years will unfold!

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O-31: Regulating the Epigenome via Chromosomal RNAs: Implications for Genome Biology and Chromosome Pathology

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It is increasingly recognized that non-coding RNAs have diverse roles in nuclear structure and genome regulation, ranging from providing a scaffold for a nuclear body to binding chromatin to regulate the heterochromatic versus euchromatic state in different cell-types. A pre-eminent example of chromatin regulation by large-non-coding RNA is *XIST* RNA, which induces silencing of one X Chromosome in mammalian female cells. It is well established that RNA from the X-linked *XIST* gene accumulates and paints the interphase chromosome territory, yet how the RNA spreads and binds in a manner restricted to the Xi nuclear territory is unknown. The answers to how *XIST* RNA interacts with the chromosome will reveal fundamental principles of chromosome architecture and its relationship to the underlying genomic sequence. In our recent studies of *XIST* RNA, we have pursued two far-reaching goals that impact distinct avenues of biomedicine. In one avenue, we have demonstrated that *XIST* RNA and X Chromosome regulation has relevance for chromosome pathology, particularly for trisomy. We have shown feasibility that *XIST* can be targeted to silence one of three Chromosome 21s in Down Syndrome patient-derived iPS cells. The broader implications of this "translational epigenetics" for Down Syndrome research will be discussed. These findings have indirect impact on a second area of fundamental biology, regarding potential roles of the high-copy repeat sequences interspersed throughout the genome. We recently provided evidence that repeat-rich "Cot-1 RNA", including L1, comprises abundant nuclear RNAs that are widespread and remain stably bound to active chromosome territories long after transcription arrest. The concept of different classes of "chromosomal RNAs" which regulate the epigenome will be discussed.

O-32: Application of the MSBWT Utility Suite to Next Generation Sequencing Datasets

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The growth in next generation sequencing output is causing an increased necessity in data structures that can store the raw genomic reads in both a compressed and accessible manner. We present a recently developed data structure called the Multi-String Burrows Wheeler Transform (MSBWT) that enables the compression of read strings from large sequencing datasets. Furthermore, the MSBWT is an indexable data structure that allows for arbitrary searches for k-mer strings in O(k) time. To demonstrate the usefulness of this data structure, we present both a downloadable MSBWT package and a series of publicly available web tools that use the MSBWT package to access raw sequencing data.

The Msbwt Utility Suite or "msbwt" package (available at https://code.google.com/p/msbwt/ or https://pypi.python.org/pypi/msbwt) provides command line functions for creating MSBWTs from raw sequencing data (FASTQ). Additionally, once an MSBWT has been generated, it provides methods for compressing and querying the data structures. The API provides many different search functions including counting k-mers, creating k-mer profiles, recovering all reads that contain a k-mer, and basic inexact matching searches. The API provides access to many other things including a simple contig building algorithm, MSBWT merge algorithm, and a special construction algorithm for long, non-uniform reads.

To demonstrate the usefulness of the MSBWT, we present a selection of web tools (accessible through http://www.csbio.unc.edu/CEGSseq/index.py?run=MsbwtTools) that all use the MSBWT to access compressed sequencing datasets. Specifically, the raw reads from all 96 samples from a three-way diallele cross involving three Collaborate Cross mouse founder strains are available through the tools. The tools allow for lookups of arbitrary k-mers and will retrieve every read containing the specified k-mer. Additional tools are provided for other genomic datasets including an inexact matching search and a pileup graph for a specified region.

O-33: Identification of core genes and networks regulating health and disease in the nervous system: a bioinformatic approach

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Many cell type-specific transcriptional profiling datasets from the mammalian nervous system are currently available, but the data have yet to be fully exploited for the identification of core genes and networks critical for influencing health and disease. We developed a multi-factorial bioinformatic approach to exploit these large datasets and identified clusters of genes that are uniquely expressed in specific neurological cell types. Their functional contribution was validated using mouse knockout and human phenotype information and pathway analysis. We showed that the use of alternative promoters in different tissue types explained phenotypic pleiotropy in some cases, opening the possibility of tissue specific drug targeting. Crucially, our analysis identified a subset of genes that were robustly implicated in neurological networks but lacked detailed functional annotation. We therefore propose that the generation and characterisation of knockout mouse mutants for these genes will identify novel candidates critical for regulating form and/or function in the mammalian nervous system.

O-34: SEC23B deficiency results in different phenotypes in humans and mice

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SEC23A/B are paralogous components of COPII vesicles, which transport proteins from the endoplasmic reticulum to the Golgi apparatus. *SEC23B* mutations in humans result in the autosomal recessive disease Congenital Dyserythropoietic Anemia type-II (CDAII). We generated SEC23B-deficient mice (B6.129-*Sec23b*$^{Gt(AD0407)Wtsi}$) and demonstrated that these animals die perinatally with pancreatic degeneration (Tao et.al PNAS). To examine the impact of SEC23B-deficiency on adult murine hematopoiesis, we harvested fetal liver cells (FLC), which contain hematopoietic stem cells during late murine embryogenesis, from SEC23B-deficient or wildtype control E17.5 embryos and transplanted them into lethally irradiated C57BL/6J mice. Recipients of SEC23B-deficient FLC did not exhibit anemia or any other CDAII characteristic. To test for a subtle hematopoietic defect, SEC23B-deficient and wildtype GFP+ FLC were co-transplanted (1:1 ratio) into lethally-irradiated recipients, with no competitive difference observed. A conditional-allele was also generated (*Sec23b*$^{tm1c(EUCOMM)Wtsi}$, *Sec23b*^{fl}). Mice with hematopoietic-specific SEC23B-deficiency, generated by crossing Tg(Vav1-cre)A2Kio into *Sec23b*^{fl} mice, exhibit no anemia. Pancreas-specific *Sec23b* knockout (using *Ptf1a*$^{tm1.1(cre)Cvw}$ or Tg(Pdx1-cre)6Tuv) generated a phenotype indistinguishable from complete SEC23B-deficiency, demonstrating that loss of pancreatic *Sec23b* expression is sufficient to explain the perinatal lethality of SEC23B-deficient mice. The SEC23B/SEC23A expression ratio was examined in murine and human tissues. This ratio is higher in mouse pancreas (12.7) compared to BM (2.6), whereas it is higher in human BM (7.8) relative to pancreas (5.5). Taken together with the high amino-acid identity between SEC23A and SEC23B (~85%), these data suggest that the tissue-specific functions of SEC23A and SEC23B have shifted during evolution between humans and mice. To determine if *Sec23a* can rescue the lethality of SEC23B-deficient mice, we have engineered *Sec23a* cDNA into the endogenous genomic locus of *Sec23b* (*Sec23b*$^{tm1(Sec23a)Dgi}$, Sec23A-B) via recombinase-mediated cassette exchange. *Sec23a* will be expressed in the same cells and at the same times and levels as wildtype *Sec23b*. A heterozygous Sec23A-B intercross is in progress.

O-35: Motile Ciliary Defects and Primary Ciliary Dyskinesia in Mice Lacking *Cfap54*

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Primary ciliary dyskinesia (PCD) is a pediatric syndrome caused by dysfunction of motile cilia and flagella that typically results in chronic rhinosinusitis, chronic otitis media, male infertility, and situs inversus, with female infertility and hydrocephalus also observed in some patients and mouse models. PCD with a decrease in ciliary motility was previously observed in mice lacking cilia and flagella associated protein 221 (CFAP221), also known as PCDP1, a ciliary protein that localizes to the central microtubule pair apparatus of *Chlamydomonas reinhardtii* flagella and forms a complex that regulates motility in a calcium-dependent pathway. Using a quantitative RT PCR approach, we show that the genes encoding the mouse homologs of the *C. reinhardtii* CFAP221 complex members are expressed exclusively in motile ciliated tissues, suggesting that they have a conserved function in motile cilia. A gene-trapped allele of one of these mouse genes, *Cfap54*, results in ciliary defects and phenotypes associated with PCD. Homozygous mutant mice have hydrocephalus and an accumulation of mucus in the sinus cavity. Tracheal epithelial cilia have a reduced beat frequency, and transmission electron microscopy reveals a defect in the structure of the C1d projection of the central microtubule pair apparatus, suggesting that CFAP54 is required for C1d assembly. In addition, male mutants are infertile due to an absence of mature sperm flagella, indicating that CFAP54 is also required for spermatogenesis. This study identifies an essential role for CFAP54 in proper assembly of mammalian cilia and flagella, and it establishes the gene-trapped allele as a new mouse model of PCD.

O-36: A mutation in the *Gnat2* locus, associated to achromatopsia, found in common albino outbred mice

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During the course of some electroretinography phenotyping studies we detected abnormal cone-derived electrical signals in common albino outbred mice (NMRI, CD1) obtained commercially from various vendors. These functional retinal abnormalities were confirmed by standard histology and immunofluorescence analyses, using optic and electron microscopy and included thickening of Bruch's membrane, multiple vacuoles in the cytoplasm of RPE cells and disorganization of the external and internal segments of photoreceptor cell layers. We decided to identify the gene responsible and the associated mutation, for the observed photoreceptor cone deficit. Various pedigrees were established and individuals were phenotyped and genotyped using the Illumina MD panel. Results indicated that the trait was monogenic, autosomal and recessive, mapping within a 34 Mb region of mouse Chromosome 3, where two-hundred genes with annotated expression in the retina were found. We tried to reduce the size of the region using SSLPs but we could not find suitable polymorphic markers in the pedigree, thus implying that the region was inherited as haplotype (identical by descent). Some of the candidate genes were directly explored by standard DNA sequencing and/or q-RT-PCR analyses with negative results. Finally, we applied NGS methods. Complete mouse exomes from mice from all three genotypes were analyzed in combination with previous genetic linkage information and we identified the gene (*Gnat2*) and the mutation (p.D200N) causing the observed phenotype. The same mutation had been identified in the ALS/Ltj mouse strain ($Gnat2^{cpfl3}$) by Chang and colleagues (IOVS, 2006), associated with type-IV achromatopsia, a rare disease causing colour blindness. We detected a substantial proportion (30-40%) of animals carrying this mutation in commercial stocks of common outbred albino mice. Therefore, these results will be of interest for anyone using these albino outbred mice for research involving retina, vision or behavioural studies.

O-37: Bioluminescence Imaging of β Cells and Intrahepatic Insulin Gene Activity under Normal and Pathological Conditions

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In diabetes research, bioluminescence imaging (BLI) has been applied in studies of β-cell impairment, development, and islet transplantation. To develop a mouse model that enables noninvasive imaging of β cells, we generated a bacterial artificial chromosome (BAC) transgenic mouse in which a mouse 200-kbp genomic fragment comprising the insulin I gene drives luciferase expression [Jcl:ICR-Tg(Ins1-luc)#Staka, (Ins1-luc BAC) transgenic mouse]. BLI of mice was performed using the IVIS Spectrum system after intraperitoneal injection of luciferin, and the bioluminescence signal from the pancreatic region analyzed. When compared with Jcl:ICR.FVB-Tg(Ins1-luc)VUPwrs mice [MIP-Luc-VU] expressing luciferase under the control of the 9.2-kbp mouse insulin I promoter (MIP), the bioluminescence emission from Ins1-luc BAC transgenic mice was enhanced approximately 4-fold. Streptozotocin-treated Ins1-luc BAC transgenic mice developed severe diabetes concomitant with a sharp decline in the BLI signal intensity in the pancreas. Conversely, mice fed a high-fat diet for 8 weeks showed an increase in the signal, reflecting a decrease or increase in the β-cell mass. Although the bioluminescence intensity of the islets correlated well with the number of isolated islets *in vitro*, the intensity obtained from a living mouse *in vivo* did not necessarily reflect an absolute quantification of the β-cell mass under pathological conditions. On the other hand, adenovirus-mediated gene transduction of β-cell-related transcription factors in Ins1-luc BAC transgenic mice generated luminescence from the hepatic region for more than 1 week. These results demonstrate that BLI in Ins1-luc BAC transgenic mice provides a noninvasive method of imaging islet β cells and extrapancreatic activity of the *i*nsulin gene in the liver under normal and pathological conditions.

O-38: Age and Sex Dramatically Affect Hyperoxic Acute Lung Injury Survival in Mice

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Mortality associated with acute lung injury (ALI) remains substantial, with recent estimates of 35-45% remaining similar to those obtained decades ago. Evidence for sex-related differences in ALI mortality is equivocal; however, death rates differ markedly for age, with ~10-fold increased mortality in older versus younger ALI patients. Mice also show significant differences in ALI mortality. To tease out genetic factors affecting survival differences, we have established a mouse model of hyperoxic ALI (HALI). Separate genetic analyses of backcross and F2 cohorts generated from sensitive C57BL/6J (B) and resistant 129X1/SvJ (X1) strains previously identified two quantitative trait loci (QTLs; *Shali1* and *Shali2)* with strong, but opposing, within-strain effects on survival time. Congenic lines confirmed these opposing QTL effects, but still demonstrated penetrance of only ~35% for resistance in 6-12 week old X1 mice. By increasing the sample size and sorting X1 control mice into 1-week age-groups, we revealed that 'age at exposure' inversely correlated with HALI survival time and could directly explain the reduced penetrance. It is well-established that newborn and very young mice are resistant to hyperoxic effects. While B-strain mice are already sensitive by 6-weeks of age, X1 mice show an extended resistance period of ~4-weeks. Importantly, this age effect on HALI survival time correlated with *Shali1* (6-week congenic mice are highly sensitive) and *Shali2* (10-week congenic mice are much more resistant). Further studies revealed significant sex-specific survival differences in subcongenics of both QTLs. Accounting for age and sex markedly improved penetrance, reduced trait variability, and helped refine *Shali1* to ~8.5Mb, and supported several sub-QTLs within the *Shali2* interval. Together, these congenics will allow studies to interrogate myriad traits during ALI development and progression, with potential to identify intermediary biomarkers that can predict outcome.

O-39: The Role of Primary Cilia in Neural Ciliopathic Disease

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Patients with mutations in genes important for primary cilia function often suffer from cognitive impairment. Mouse mutants in several ciliary genes have forebrain phenotypes. The underlying mechanisms of these phenotypes are not fully understood. We have previously demonstrated loss of *Ttc21b* leads to decreased retrograde trafficking in the cilium. A null mouse allele (*Ttc21b*^{aln}) shows microcephaly and abnormal patterning of the embryonic neural tissue along both the anterior-posterior and dorsal-ventral axes. However, important aspects of the *Ttc21b* null forebrain phenotype remain completely unexplored. Here we show abnormal proliferation, migration and differentiation of neural progenitors. We are also using a conditional allele to determine the spatio-temporal requirement for *Ttc21b* (*Ttc21b*$^{tm2a(KOMP)Wtsi}$). Surprisingly, a forebrain specific ablation has only minor consequences for cortical development. Our current model holds the *Ttc21b* null phenotype is due to an early event in patterning the nascent neural ectoderm, prior to definitive forebrain development. The consequences of the loss of *Ttc21b* then perdure into the neurogenic cortex. We are comparing these results from the *Ttc21b* mutants to conditional ablations of *Kif3a* (*Kif3a*^{tm2Gsn}) and *Ift88* (*Ift88*^{tm1Bky}). Using a series of cre transgenic mouse lines to define the spatiotemporal role of these genes, we note that each gene has unique phenotypes from ablation at specific time points. Taken together, these studies are revealing subtle differences in the role of ciliary proteins in the control of neural patterning and neuroprogenitor proliferation/differentiation. We are also exploring the molecular mechanisms of *Ttc21b* activity. We demonstrate a genetic interaction with a null allele of *Gli3* (*Gli3*$^{Xt-J}$) and rescue of some *Ttc21b* phenotypes with an allele of *Gli3* lacking a putative transactivator domain (*Gli3*^{tm1Urt}). We also show a biochemical interaction with GLI3. These are consistent with a model where GLI3 is a cargo protein linked to the ciliary retrograde machinery by TTC21B.

O-40: Discovering genetic modifiers of Niemann-Pick disease, type C

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Niemann-Pick disease, type C (NPC) is a fatal neurodegenerative disorder that exhibits intracellular accumulation of unesterified cholesterol and glycosphingolipids. Mutations in *NPC1* account for 95% of NPC patients, however disease severity varies independent of specific mutation status. For example, patients harboring homozygous mutations for the common I1061T hypomorphic *NPC1* allele exhibit onset of neurological symptoms from 3 to 15 years. This phenotypic variability along with a small patient population severely limits linkage analysis for modifiers, thus we currently have little understanding of how genetic variation contributes to phenotypic variation.

To identify genes modulating NPC severity, we developed a cellular assay using Lysotracker Red (LTR) staining of NPC patient-derived fibroblasts and found a direct correlation with the time of onset of neurological symptoms. Using this assay, we completed a high-throughput siRNA-based screen using an arrayed siRNA library targeting 10,415 genes in the human druggable genome. Candidate modifiers were selected for suppression of the NPC phenotype, as measured by reduced LTR staining, and a secondary screen is in progress to confirm a ≈250 gene subset using additional siRNAs. In addition, candidate modifiers are being identified in a mouse model for NPC disease that harbors the NPC1 I1061T mutation at the endogenous *Npc1* locus (*Npc1*$^{tm1(NPC1)Ory}$). We have observed significant phenotypic differences between the BALB/c background and C57BL6/J background. Metabolomics profiling on intercross animals is being used for linkage analysis to identify strain-specific variations for further analysis.

Candidate genes from these two modifier screens will be assessed in a variety of assays including filipin and LAMP1 staining to evaluate their ability to ameliorate NPC subcellular phenotypes. The most promising candidates will then be evaluated *in vivo* using CRISPR/Cas9-mediated targeting to assess their effect on phenotypes of NPC mouse models, allowing these genes and their associated pathways to be assessed for pharmacological treatments.

O-41: Community annotation of the rat genome

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The rat is an important model organism for various fields of research, such as physiology, neurology and behaviour. To link this phenotype based research to genotype, a reliable gene set is essential. Following a rat genome workshop at the Institute in March 2013, which brought together researchers, developers, curators and the Wellcome Trust Sanger Institute's HAVANA team, we have taken on the annotation of the Norway Brown rat reference genome. Our rat annotation is done at the behest of the scientific community, members of which can nominate genes, families and regions of interest at rat-annotation@sanger.ac.uk. As with all genome annotation, nomenclature plays an important role, as it helps point to function and to orthology and paralogy relationships. To that end we closely collaborate with the rat genome database (RGD) and the human gene nomenclature committee (HGNC).

The HAVANA group manually annotates vertebrate model organism genomes to a high degree of accuracy and comprehensiveness and with a very fine-grained biotype ontology. We can now present around 1200 genes, including protein-coding genes, pseudogenes and lncRNAs, on the RNO 5.0 assembly. The gene number will continue to grow and we will be employing comparative annotation to map the MHC region, adding the rat MHC to the growing roster of HAVANA annotated MHC regions that includes human, mouse and pig. All annotation is available through the VEGA genome browser and as part of a merged gene set in the Ensembl genome browser.

The rat genomics community will meet again during the Rat Genomics and Models conference on 1-4 December at the WTSI.

I will present an overview of the rat genome community annotation project and highlight challenging examples inherent in annotating whole genome shotgun sequence and show how gene annotation helps identify genome assembly issues.

O-42: Interspecific Recombination Between Orthologous Human and Mouse BAC Clones in *E. coli*: Exploring Scalable Humanization of Cancer-Relevant Genes in the Mouse Genome

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Here we assess the feasibility and scalability of using standard recombineering techniques to humanize large segments of mouse genome, modeling a leukemia-related deletion polymorphism of the human *BCL2L11* (*BIM)* gene as a test case. Using a combination of two recombineering-based insertion events and two gap-repair-enabled retrieval steps, we have assembled a targeting vector with 26-kbp of the human *BCL2L11* gene (encompassing exons 2 through 4) flanked by 12-kbp and 26-kbp mouse homology arms, respectively. FLP and phiC31 recombinase-binding sites flank each of two selectable marker cassettes at the ends of the humanized segment and a pair of loxP sites will be used to conditionally delete a region of intronic DNA missing in the human disease-related variant. Successful transfer of the humanized region into mouse embryonic stem (ES) cells by homologous recombination will create a strain of mice carrying the humanized region. Once completed, the humanized mouse strain will model a deletant form *BCL2L11* carried by 12% of the Asian population and associated with treatment-resistant chronic myeloid leukemias and lung cancers. Once reduced to practice, our process can be expanded to include other cancer-related genes providing a resource to scientists studying the pathological activities of human genes or developing therapeutics in a whole animal context. This presentation also describes our development of a novel parallel engineering technique tentatively named *trans*-recombineering.

O-43: Dissection of expression QTL in Diversity Outbred mice.

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The emergence of high throughput sequencing (HTS) technologies has coincided with the development of advanced mapping populations including the Diversity Outbred (DO) mouse heterogeneous stock. The application of HTS to the DO has the potential to provide nucleotide resolution of causal variants underlying phenotypic differences, however the increase in information content comes with increased analytical complexity. We have developed novel methods to exploit the genetic diversity and heterogeneous diploid genomes of the DO to reveal new layers of information and inform fine mapping of phenotypic and expression QTL (eQTL).

We profiled the liver transcriptome by RNA-seq in 469 DO mice and estimated gene, isoform, and allele expression. We identified 10-fold more significant local eQTL than distant eQTL, showing that most of the variation in liver transcript abundance in the DO derives from segregating local genetic variation. Founder strain differences in allelic expression estimates confirmed that cis-acting mechanisms underlie most local eQTL, and DO allele estimates correlate well with gene expression in the founder strains. Most eQTL appear biallelic suggesting a single causal variant, however complex 3- and 4- allele patterns are observed. Cis-eQTL with allelic expression patterns that deviate from the known strain ancestry are most amenable to fine mapping, and in some cases we predict a single causal variant. Distant eQTL with large effects are rare, however we have developed methods to amplify trans effects and systematically test candidate regulatory genes in the interval. All eQTL data and analyses are publicly available as an interactive web application at do.jax.org.

O-44: Unbiased HIGH-THROUGHPUT mouse TISSUE phenotyping REVEALS NOVEL candidate GENES for human pathologies

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The Sanger Institute's Mouse Genetics Project (MGP) aims to make a significant impact on our understanding of gene functions and their role in disease by generating, characterising and archiving 160 lines of knockout mice per year. Knockout mice are phenotyped using a battery of tests relevant to important biological areas including metabolism, sensory, immune disorders and motor function. The breadth of our phenotyping platform generates phenotypic data on a spectrum of disease conditions. Here we report on the benefit of adding tissue-focused screening to our high-throughput approach.

Tissues for *ex-vivo* analysis are collected at 16 weeks of age and sent to area experts for in-depth, specialist analysis. Our tissue-focused collaborations include eye histopathology, skin analysis via tail epidermis wholemount, brain histopathology and the origins of bone and cartilage disorders (OBCD) project. We collect eyes and brains from 3 males; and tails and specific bones from 2 females for each mutant line, regardless of prior knowledge or expectation, to find novel phenotypes in genes of which nothing is known or pleiotropic ones that are known for an unrelated phenotype.

Here we present some examples of the added value these tissue-focused collaborations bring to the project. These include $\textit{Slc9a8}^{tm1a(KOMP)Wtsi}$ for eye histopathology, $\textit{Mysm1}^{tm1a(KOMP)Wtsi}$ for tail epidermis wholemount, $\textit{Ropn1l}^{tm1b(EUCOMM)Wtsi}$ for brain histopathology and $\textit{Prpsap2}^{tm1a(EUCOMM)Wtsi}$ for the OBCD project.

These genes provide candidates for further study of their biological relevance, both in mice and in humans.

O-45: Exome sequencing reveals pathogenic mutations in 84 strains of mice with Mendelian disorders

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The advent of high throughput exome sequencing has enabled unprecedented capacity for Mendelian disease gene discovery and heralded a resurgence in the value of phenotype driven approaches, wherein spontaneous alleles in model organisms often provide a more complete recapitulation of disease gene function than can be provided by null alleles alone. By harnessing these technologies, we sought to identify pathogenic variants in a large collection of spontaneously arising mouse models of Mendelian disease. We have developed an exome analytics pipeline that is optimized for mouse exome data and created a variation database (http://mmrdb.jax.org) that allows for reproducible, user defined data mining as well as nomination of mutation candidates through knowledge-based integration of sample and variant data. Using these new tools, pathogenic mutations were nominated and validated for nearly 50% of the strains in our study, providing novel gene phenotype links, novel alleles of known genes and using publicly available data sharing resources, new human disease associations. Despite the increased power offered by unlimited pedigrees and controlled breeding, over 50% of our exome cases remained unsolved. Using manual analysis of exome alignments and whole genome sequencing, we provide evidence that significant fraction of exome recalcitrant mutations are structural mutations involving coding sequence.

O-46: PopulASE: A Tool for Estimation of Isoform-specific and Allele-specific Expression with RNA-seq Data from a Genetically Diverse Population

Kwangbom Choi[*], Narayanan Raghupathy, Steven C Munger, Daniel M Gatti, and Gary A Churchill

The Jackson Laboratory

The Diversity Outbred (DO) mouse population is a new heterogeneous stock derived from the eight Collaborative Cross (CC) founder strains. The DO mice have uniformly high levels of heterozygosity and genetic diversity, and thus provide a high-resolution mapping resource for identifying key genetic factors underlying complex traits and disease. As each DO's genome is a unique, pseudo-random mosaic of eight inbred strains, estimating allele specific expression (ASE) from the reference alignment is computationally challenging and error-prone. We would have to visit every mismatch in the billions of reference alignments, refer to the known strain variations, and evaluate which strain each read originates from. Misalignment due to unaccounted strain variations in orthology and paralogy space is also common.

For more accurate estimation of ASE in the DO, we first created eight custom genomes by incorporating strain-specific SNPs and short indels into the reference genome. We then aligned each read against all eight alleles of transcript sequences and stored only the best alignments. Next, we estimated diplotype probability at all the transcript loci by interlinking genotyping array information at selected markers and "8-way" read alignment profile at each transcript with a hidden Markov model. Finally, we integrated the diplotype probability and the read alignment profile using the Expectation-Maximization (EM) algorithm and derived the best posterior probability that describes the strain origin of each read. In the EM process, we were able to further improve the ASE quantitation by borrowing information of each transcript from across samples. The core idea is to refer to how each transcript behaves at the population level and adjust (or shrink) sample-level abundance estimates accordingly. The degree of shrinkage would be lower by design when there exist higher level of dispersion since data tells us that each individual is less likely to follow a single population-level distribution in such cases.

O-47: The Mouse Genomes Project: From variants to genomes

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The Mouse Genomes Project is an ongoing effort to sequence the genomes of the common laboratory mouse strains, cataloguing all forms of molecular variation, and to produce genome sequences of each strain. Phase 1 (2009-2011) of the project involved deep sequencing of 18 strains and creating comprehensive catalogs of molecular variation ranging from single base changes up to large structural differences. We are now in phase 2 (2012-) of the project with the focus being the production of accurate genome sequences and strain specific gene annotation for 16 strains (129S1/SvImJ, A/J, ARK/J, BALB/cJ, C3H/HeJ, C57BL/6NJ, CAST/EiJ, CBA/J, DBA/2J, FVB/NJ, LP/J, NOD/ShiLtJ, NZO/HlLtJ, PWK/PhJ, SPRET/EiJ, and WSB/EiJ). We resequenced each of the strains to between 40-80x coverage on the HiSeq platform, generated multiple large fragment libraries of varying sizes (3-40Kbp), longer PacBio sequencing and optical maps. In our most recent release of we have reached scaffold N50 lengths of 0.2-0.8Mbp. By comparing the de novo assembly of C57BL/6NJ to GRCm38, we can assess the chromosome scaffold accuracy and find the scaffolding error rate to be 0.2%. Our initial assessments of the genome sequences show that they contain 98-99% of the protein coding exons with 60-72% of genes currently contained in single scaffolds. We have examined the representation of 742 validated structural variants in the assemblies and find that the majority are represented correctly. We have created the first round of the strain specific automated gene sets using a combination of Illumina RNA-Seq sequencing and the reference genome annotation. We highlight some interesting cases of genes with differing gene structures compared to the C57BL/6J reference annotation.

O-48: Collaborative Cross founder strains: A comprehensive and comparative phenotypic analysis at the German Mouse Clinic

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The Collaborative Cross (CC) is a large panel of mouse-inbred lines that derived from eight founder strains, including three wild-derived strains (A/J, C57BL/6J, 129S1/SvlmJ, NOD/ShiLtJ, NZO/HILtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ). A comprehensive and comparative phenotyping screening was carried out at the German Mouse Clinic (GMC, www.mouseclinic.de) to identify phenotypic differences and similarities between the eight founder strains. In total, more than 550 parameters were tested including allergy, behavior, cardiovascular parameters, clinical chemistry, dysmorphology, bone and cartilage, energy metabolism, eye and vision, immunology, lung function, neurology, nociception, and pathology. For each strain, at least ten animals were phenotyped resulting in highly robust data sets. Metadata analysis showed that the three wild-derived strains are most different from the laboratory strains and for some parameters the wild-derived strains are more similar to each other than to the laboratory strains. These strain-specific traits were observed for both genders of the founder strains. Hierarchical cluster analysis showed that NZO/HILtJ, A/J and 129S1/SvlmJ were most different from all other strains. The analysis of screen-specific parameter sets recapitulated the global tendency of phenotypic similarities between laboratory strains, but also detected phenotypes that distinguished a single strain from all others. For example in the cardiovascular screen PWK/PhJ was found to have the most distinct cardiac phenotype. Our comprehensive studies highlight the value of the founder strains and upcoming CC resource for phenotype-genotype associations for many genetic traits that are highly relevant for human diseases.

O-49: Spontaneous Mutation Discovery in Mouse Models of Mendelian Disease: Using RNA-Seq to Identify Non-Exomic Mutations

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Advances in high-throughput sequencing technology have rapidly accelerated the discovery of rare variants underlying Mendelian disease. Whole-exome sequencing (WES) alone has uncovered over 100 mutations causing these disorders in human patients. Despite these significant advances, causative gene discovery by WES still hovers around 50% indicating the complex nature of naturally occurring, spontaneous mutations. At The Jackson Laboratory's Mouse Mutant Resource (MMR), our success rate for spontaneous mutation discovery, despite the potential advantages provided by unlimited pedigrees and genetic mapping, closely mirrors that of clinical WES efforts for Mendelian diseases. We propose that mutations that escape detection by whole-exome sequencing are structural in nature, or are non-exomic, lying outside a gene's coding region in enhancer or regulatory regions. Through the use of genetic mapping and RNA-Sequencing (RNA-Seq), we have investigated a small subset of strains harboring mutations that were undetected by WES and have successfully nominated candidate genes likely to be causative of their disease phenotypes. Here we present examples of these mutations and demonstrate that RNA-Seq is a valuable tool for revealing genes that underlie Mendelian disease. In addition to directing mutation discovery efforts, this method provides critical information about regulatory architecture and structural mutations and will inform optimization of future algorithms and WES pipelines.

O-50: Collaborative Cross founder strains – Phenotyping at the German Mouse Clinic: Known and newly identified features of strain - clinical chemistry and hematology

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As part of a comprehensive comparative phenotyping of the eight parental strains used to produce the Collaborative Cross (CC) lines (A/J, C57BL/6J, 129S1/SvlmJ, NOD/ShiLtJ, NZO/HILtJ, PWK/PhJ, WSB/EiJ, and CAST/EiJ) carried out at the German Mouse Clinic (GMC, www.mouseclinic.de) we have measured a broad spectrum of clinical chemistry parameters in plasma samples and basic hematological parameters in EDTA-treated whole blood samples. Furthermore, intraperitoneal glucose tolerance tests (IpGTT) and analysis of fasting plasma lipid and glucose values have been applied for a subset of five strains (A/J, C57BL/6J, 129S1/SvlmJ, NOD/ShiLtJ, NZO/HILtJ). Evaluation of the results revealed both, strain specific phenotypes that have previously been described in publications and were confirmed by our findings as well as strain specific characteristics that have not been described yet. For example in A/J mice we found very low fasting cholesterol levels, but high fasting glucose levels and high plasma phosphate concentrations compared to the other strains tested. NOD/ShiLtJ mice showed the well-known development of hyperglycemia due to autoimmune destruction of beta cells mainly in females, and were further characterized by low red blood cell counts accompanied by high mean corpuscular volumes for erythrocytes and platelets. And in NZO/HILtJ mice we found high plasma albumin levels and alpha-amylase activities, besides described dyslipidemia. Mice of the three wild-derived strains PWK/PhJ, WSB/EiJ, and CAST/EiJ shared many characteristics besides the low body weight. Specific features are for example low plasma alpha-amylase activity in PWK/PhJ females and high ALP activity in PWK/PhJ males, low plasma albumin levels in CAST/EiJ mice and WSB/EiJ males as well as low plasma triglyceride values in WSB/EiJ mice of both sexes.

O-51: The Mouse Gene Expression Database (GXD)

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The Gene Expression Database (GXD) is an extensive resource of mouse expression information. It integrates data from RNA in situ hybridization, immunohistochemistry, in-situ reporter (knock-in), RT-PCR, northern blot, and western blot experiments with a primary focus on mouse development, covering all developmental stages and organ systems and comprising data from wild-type and mutant mice. As an integral part of the larger Mouse Genome Informatics (MGI) resource, GXD combines its expression data with genetic, functional, phenotypic, and disease-oriented data. In recent years, GXD has grown tremendously in terms of data content and search utilities. Through curation of the literature, electronic data submissions, and collaborations with large-scale data generators, GXD has acquired nearly 1.5 million expression results from over 67,300 expression assays for more than 13,800 genes, including expression data from nearly 2,000 mouse mutants. In addition, the database contains over 262,000 expression images. GXD annotates its data, including these images, with extensive metadata such as the genes analyzed, probes used, strain and genotype of the specimens, and developmental stages and anatomical structures in which expression was analyzed. GXD thus enables thorough data integration and allows users to search for expression data and images in many different ways, using a variety of biologically- and biomedically-relevant parameters. Recently, we have made many improvements to the search interface, such as: implementing new types of data summaries; adding data filters to iteratively refine search summaries; enhancing the anatomy ontology and building a new anatomy browser; and making data available through MouseMine. Our newest database release includes interactive tissue-by-developmental stage and tissue-by-gene matrix views of expression data. These matrices provide users with intuitive high-level summaries of expression data from where they can move to levels of greater detail (see also abstract by Forthofer et al.). Visit GXD at www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD062499.

O-52: The JAX KOMP2 Program: functional annotation of the mammalian genome

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The overarching goal of the Knockout Mouse Phenotyping Program (KOMP2) and its partners in the IMPC International Mouse Phenotyping Consortium (IMPC) is to generate and phenotype a genome-wide set of knockout mice to build an encyclopedia of gene function. By applying a standardized set of broad phenotyping modalities for these mice, KOMP^2 eliminates the variability and ascertainment bias intrinsic to analysis in individual labs, thus maximizing the phenotypic data available for each strain. Now more than halfway through our ultimate goal of producing 833 knockout strains, JAX has built and implemented a high-throughput pipeline of 25 tests comprising several hundred phenotypic parameters to characterize adult mice. To properly capture the rich functional information provided by embryonic lethal strains, we have added a mouse embryo phenotyping pipeline, which includes the use of high-resolution 3D imaging for high-throughput generation of rich datasets. Thus far, JAX has observed identified more than 60 knockout lines that display combined partial and complete lethality, including both novel genes and novel findings for previously published gene knockouts. By using the LacZ reporter provided by the KOMP2 alleles, we are assembling a comprehensive atlas of gene expression, revealing numerous novel gene expression patterns in both embryonic and adult tissues. All of the data is made publically available as it is generated at www.mousephenotype.org. To take advantage of the paradigm shift driven by developments in CRISPR-based genome editing, we have piloted its use to generate knockout mice for nearly 40 gene targets, providing key data for optimization of design, screening techniques and assessment of off target mutagenesis risk. Additional developments in the generation of knockout, knock-in and other allele designs will be presented. With the scale and throughput of this platform in place, we will look towards its current and future application for modeling novel human disease variants.

O-53: Potential Driver Genes of Ovarian Carcinogenesis identified by Sleeping Beauty Mutagenesis

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Serous epithelial ovarian cancer (SEOC) is the most lethal gynecological malignancy in women. Germline mutation of *BRCA1* is the best known risk factor for developing SEOC, while somatic mutation of *TP53* is the most common genetic change in SEOC. However these changes individually, or combined, are insufficient to induce SEOC in mouse models, suggesting that additional unknown genetic factors are required for ovarian carcinogenesis. To identify these additional genetic factors we utilized Sleeping Beauty (SB) insertional mutagenesis.

The following genetically engineered mice were cross bred: homozygous floxed *SB* ($SB^{flox/flox}$: STOCK TgTn(sb-T2/Onc2)6113Njen/Nci Gt(*ROSA)26Sor*$^{tm2(sb11)Njen}$), homozygous floxed *Brca1* knock-out ($Brca1^{flox/flox}$: STOCK $Brca1^{tm2Brn}$) and *Trp53* mutant (Trp^{mut}/+: B6.129S2-$Trp53^{tm1Tyj}$/J (Stock No. 002101)). To delete *Brca1* and activate SB mutagenesis in the ovarian surface epithelium, adenoviral cre recombinase was injected under the ovarian bursal membrane of mice. Tumors were assessed for SB transposase activity by immunohistochemistry. DNA extracted from ovarian tumors underwent high-throughput sequencing for T2/Onc2 insertion sites (Illumina, University of Iowa).

Ovarian tumors were observed at low penetrance from 30 weeks post-surgery in $SB^{flox}/+Trp53^{mut}/+$ mice and $SB^{flox}/+Brca1^{flox/flox}Trp53^{mut}/+$ mice. No tumors were observed in $SB^{flox}/+Brca1^{flox/flox}$ or $SB^{flox}/+Brca1^{flox}/+$ mice. Sequencing of insertion sites identified the mutated genes, 67 of which were also altered in 10 - 30% of human cases from The Cancer Genome Atlas SEOC dataset (N = 316). This gene-set was enriched for kinases including *Wnk1*, *Dyrk1a* and *Gsk3b,* and small GTPase regulators including *Smap2, Trio* and *Dock10*. Other genes of interest included tumor suppressor genes *Wwox, Arid1b* and *Cdh4,* and the transcriptional regulator, *Zfat.*

This screen identified novel potential driver genes of ovarian cancer. In addition, genes previously associated with ovarian cancer were identified, providing proof of principle for this approach. Investigation of several of these novel genes is currently underway and will lead to further insights into the pathogenesis of ovarian cancer.

O-54: The importance of gene annotation in genome manipulation

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Our ability to manipulate genomes has undergone a recent revolution with the establishment of CRISPR-Cas9 technologies. With nearly 300 million CRISPR sites in human and mouse, potential binding sites for guide-RNAs should occur on average every 10 bases. In practice however, the frequency of CRISPR sites varies with GC content, which combined with possible off-target binding of the guide-RNAs can impose limitations. The propensity of guide-RNAs to misalign at off-target binding sites presents further challenges for the large-scale mapping of CRISPR sites. This has led to the establishment of online portals for the searching and analysis of CRISPRs, including the WTSI Genome Editing (WGE) site (http://www.sanger.ac.uk/htgt/wge). Although, such portals facilitate the identification of CRISPR sites for genome editing, additional factors must be considered when evaluating CRISPRs and potential off-target binding in a species-dependent manner. In particular, human variation may have a significant impact on the efficacy of guide-RNAs in both human iPSCs and, ultimately, therapeutic situations. The impact of off-target effects should also be considered within a wider genomic context that includes non-coding genes and other regulatory elements.

It is within this wider context that the HAVANA group contributes to the analysis and evaluation of CRISPR resources. Until recently, annotation of the mouse genome has predominantly been focused on protein coding genes; as such pseudogenes and non-coding regions are poorly represented. As part of GENCODE, we have a mandate to annotate mouse genes to the same standard as the human gene set, including experimental validation. It is this annotation that forms the basis of the GENCODE gene-set that is displayed in both the ENSEMBL and UCSC genome browsers. With our expertise in the analysis and annotation of the human and mouse genomes, I will highlight the importance of high-quality genome annotation in determining genome-editing strategies using CRISPRs.

O-55: A conditionally inducible dominant negative allele of *Sox10* for analysis of gene function in multiple neural crest lineages

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Neural crest-derived neurons and glia are critical for normal development and function of the peripheral nervous system. *Sox10* is a transcription factor that is essential for development of a wide range of neural crest lineages including many types of peripheral glia like Schwann cells and satellite glia, neurons and glia of the enteric nervous system, melanocytes in the skin, craniofacial bones, teeth, and the adrenal medulla. Investigating *Sox10* function in these distinct lineages has been difficult because simple gene knockouts cause complete loss of gene expression in early neural crest progenitors resulting in embryonic lethality. Efforts to temporally induce loss of *Sox10* have been hampered by kinetics of mRNA and protein decay. We have generated a COnditional INducible ("COIN") dominant negative allele of *Sox10* (*Sox10$^{tm1.1Sout}$* or *Sox10*-COIN) in mice as a novel tool for analysis of gene function. Mice bearing a COIN cassette in the *Sox10* locus are designed to be phenotypically normal until cre action flips a fluorescently tagged dominant negative Sox10 isoform into the coding frame of exon four. Our initial characterization indicates that the COIN allele functions as a dominant negative inhibitor of transcription and appropriately labels neural crest derivatives with a fluorescent reporter following cre action. Crosses of *Sox10*-COIN mice with cre drivers *in vivo* produce progeny that exhibit pronounced deficits of peripheral glia and intestinal phenotypes as well as marked hypopigmentation. Current efforts are specifically focused on defining the effects of the *Sox10*-COIN allele on oligodendrocyte, peripheral glia, and enteric neural crest-derived lineages before and after COIN inversion. The ability to conditionally disrupt *Sox10* expression and function in distinct neural crest lineages opens avenues for analysis of developmental processes that are relevant for directed differentiation of progenitors cells to treat central and peripheral neuropathies.

O-56: Impact of repetitive element transcriptional changes in cocaine addiction

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Expression of repetitive elements (REs) can interfere with transcription of genes located in their immediate vicinity. For instance, we have shown that normal regulation of the *a* nonagouti (agouti) gene in mice can be hijacked by the promoter of an intracisternal A particle (IAP) inserted within the first intron of the gene. The result is a chimeric RNA transcript containing the IAP sequence followed by the normal agouti message. Such ability of the IAP to control agouti expression is dependent upon the epigenetic status of the IAP: methylation of this element effectively silences its expression and its genetic control of agouti. We hypothesized that RE cis-regulation of downstream genes is more common than initially thought. We developed a bioinformatics framework to study the expression of all classes of REs and to find fusion transcripts between individual RE and a closely linked non-repetitive gene along the chromosome. We used RNA-seq data sets derived from the nucleus accumbens (NA) of cocaine-treated and control mice as we had previously shown that repeated cocaine exposure leads to the de-repression of several REs. Our results show that ~805K RE loci are expressed in the NA, from which 2,500 are differentially regulated by cocaine. Notably, we found chimeric reads between a RE and an adjacent non-RE sequence in 490 genes, many of which were confirmed to be expressed *in vivo* using RT-PCR. Importantly, we found that one of the identified genes significantly diminishes cocaine-reward behavior *in vivo*, but only when expressed as a chimeric transcript. Collectively, these data indicate that cocaine dramatically changes the transcriptome of the NAc through changes in the expression of REs and the formation of chimeric transcripts. *This research was supported in part by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.*

O-57: Allele-specific knock-down of *KRAS* mutations in cancers by using a novel alkylating Pyrrole-Imidazole Polyamide (KR12)

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Despite extensive efforts to develop chemotherapeutic drug(s) targeting mutated *RAS*, the successful drug discovery, especially targeting *KRAS* codon 12 mutation, has never been made. We have found that Pyrrole-Imidazole polyamide seco-CBI conjugate (KR12) selectively recognized mutated *KRAS* sequence (ACGCCT-A/T-CA) at codon 12 and significantly suppressed tumor growth specifically in human colon cancer cells with G12D or G12V mutation. *KRAS* expression suppressed preferentially at mutated allele and active KRAS were markedly reduced. G2/M arrest, senescence and subsequent apoptosis by activating the p53 pathway were observed in KR12-exposed LS180 cells with G12D heterozygous mutation. In LS180 and SW480 (G12V homozygous mutation) xenograft colon cancer models, KR12 treatment induced massive tumor growth suppression with low host toxicity. Collectively, our current results strongly suggest that KR12 is a specific alkylating agent against *KRAS* codon 12 mutations, and could become a powerful candidate compound for the *KRAS*-mutant tumor treatment.

Poster Abstracts

P-01: A T to C mutation in the conserved spacer region in one of the loxP sites inhibits the cre recombinase activity *in vivo* but not *ex vivo*.

(See abstract SO-01 in the student/postdoc session)

P-02: Neuroscience Research Tool Strains in the JAX Repository

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The Jackson Laboratory (JAX) Repository distributes mouse lines engineered with recently developed optogenetic and calcium-sensing technologies directed to defined cell populations by specific promoters, and by cre recombinase and/or by tet-on/-off technologies.

Opsins are light-activated proteins that alter membrane potential in neurons, so that stimulation with light allows rapid control of neuronal activity. Several strains express next generation improved/optimized opsins fused to fluorescent proteins, including archaerhodopsin, channelrhodopsin and/or halorhodopsin variants.

GCaMP proteins exhibit fluorescence in response to calcium binding, thus serving as an indication of neuronal activation. Mouse models expressing optimized GCaMP2, GCaMP3 and GCaMP6 variants are available.

Several strains combine cre-lox and tet-on/-off functionality. After removal of an upstream floxed-STOP, such mice allow tet-dependent expression of optimized channelrhodopsin (Chronos/EGFP), halorhodopsin (Jaws/EGFP) or GCaMP6f. Similarly designed mice have cre- and tet-dependent expression of a voltage-sensitive FRET chromophore pair upon membrane depolarization. This set features models from the Allen Institute for Brain Science, the GENIE project (Janelia Farm/HHMI), Duke/MIT and others.

Mice with pharmacogenetic receptors and photoactivatable fluorescence add to the collection of tool strains available, including those allowing individual cell labeling with nuclear-localized, membrane-targeted or cytoplasmic fluorescent proteins following cre recombination.

The JAX Repository is a centralized facility for mouse models development, rederivation, distribution and cryopreservation. Hundreds of new strains are added annually to one of the largest collections of characterized mouse strains available. Repository holdings may be searched online (JAXMice database: **jaxmice.jax.org/query**). Researchers wishing to donate their mouse strains may use our online submission form (**jax.org/donate-a-mouse**). The JAX Repository is supported by NIH, The Howard Hughes Medical Institute and several private charitable foundations.

*Please stop by for a detailed list of recently added models. Please also visit our resources for Optogenetics (**research.jax.org/grs/optogenetics.html**), Cre-dependent Optogenetic Tools (**jaxmice.jax.org/list/ra2600.html**) and Cre Strains for Neurobiology (**cre.jax.org/NeuroCres.html**).*

P-03: Generation of albino C57BL/6 mice with G291T mutation in the tyrosinase gene by the CRISPR/Cas9 system

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The genome editing with engineered nucleases is an advanced technology for producing gene modified animals by directly injecting DNA or mRNA of site-specific engineered nucleases into the one-cell embryo. In genome editings, designation and construction of CRISPR/Cas9 system are easier than the others, moreover, when co-injection of CRISPR/Cas9 and single strand oligo DNA donor (ssDNA) is performed, it is possible to induce targeted small-scale gene mutation. Single nucleotide polymorphisms (SNPs) and mutations (SNMs) are associated with a variety of human diseases. However, creation of SNMs-induced mice is difficult. In this study, we try to produce albino C57BL/6 mice by CRISPR/Cas9 induced SNM in the *Tyr* gene. The *Tyr* gene codes for enzyme tyrosinase that is necessary for melanine production. A couple of SNMs (G291T, G369C) in the *Tyr* are the causes of albino phenotype in mice. To induce the SNM G291T in the *Tyr*, we injected CRISPR/Cas9 expression DNA vector and mutant ssDNA (G291T in the *Tyr*) into 224 one-cell embryos from C57BL/6J mice. As a result, 60 mice were obtained and 28 of which showed ocular albinism and absence of coat pigmentation. Genomic sequencing analysis of the albino mice revealed that target of SNM, G291T in the *Tyr* gene, occurred in 11 mice and one founder homozygously mutated ($Tyr^{em2Utr}/Tyr^{em2Utr}$). The remaining albino founders without *Tyr* G291T mutation also possessed biallelic deletion and insertion mutants adjacent to the target site in the *Tyr* locus. These results suggest that disease model animals could be generated by CRISPR/Cas9 mediated SNMs.

P-04: The CrePortal (www.creportal.org), a resource for conditional mutagenesis in the mouse

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Conditional mutagenesis is a powerful technique for interrogating gene function in a cell- or tissue-specific manner, potentially with a defined temporal component. To exploit fully the available ES cells or mice bearing conditional-ready mutations (e.g., from the International Mouse Knockout Consortium), information about spatial/temporal specificity of recombinase alleles is essential to select driver/allele combinations best suited to experimental work. The CrePortal (www.creportal.org) resource focuses on recombinase tools (primarily cre) generated by research labs and large cre toolsets created by initiatives such as the NIH Neuroscience Blueprint Cre Driver Network, Allen Institute for Brain Science, Pleiades Promoter Project, and EUCOMMTOOLS. Through integration with the Mouse Genome Informatics resource (MGI, www.informatics.jax.org), the CrePortal provides a central access point to existing recombinase alleles and curated data about them. Important new enhancements improve searching, provide summary overviews, and allow users to directly submit data or add observations about recombinase alleles used in their research. A new implementation of the anatomy search allows querying for cre activity in specific anatomical substructures (e.g. atrium cardiac muscle) using the Mouse Anatomical Dictionary with the systems containing matching annotations highlighted in the summary. Querying by a specific driver is an alternate search method. Results display a summary of recombinase alleles with activity (observed or absent in a specified structure) or all alleles with a particular driver, and include information about the driver/promoter, inducibility, associated references, and public repository availability. Allele symbols link to a recombinase data overview in matrix format with each cell providing activity presence/absence data for specific anatomical system by age combinations and links to further details and images. Anatomical systems can be expanded to view substructures with annotations. As of July 2014, the CrePortal contained detailed information about >2180 recombinase alleles that utilize >700 distinct drivers/promoters. Supported by NIH grants OD011190, HG000330, HD062499.

P-05: Detecting cellular response to drugs in mammalian cells

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Detecting a dynamic change of the molecular network in cells is essential for understanding mechanism of cellular responses to stimulus. We use CAGE (Cap Analysis Gene Expression), a technology to capture 5'-end of RNA, and Next Generation Sequencing to detect transcription start sites of mRNAs as well as noncoding RNAs and inferring promoters and their activities. Previously, we have detected differences of transcripts quantitatively by CAGE sequencing, and inferred key regulatory factors and their edges contributing to cell differentiation[1]. We also use CAGE for quantitative profiling of drug responses of mammalian cells. In the study, we succeed to identify a distinct set of promoters affected by low doses of drugs on signal-transduction pathways, and thus show sensitivity to a weak inhibition of the pathways[2]. We are now developing a pipeline to detect differences of drug action among cell-types and among drugs. We detect differences or similarities of cellular responses between gene-based and drug-based perturbation and predict druggable targets in cells.

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P-06: Applied Genome Editing with CRISPR/Cas9 Plasmid in Mice

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The CRISPR/Cas9 system has been developed as a genome editing method. The CRISPR/Cas9 plasmid DNA vectors are very easily constructed. In addition, gene modified mice can be simply produced by microinjecting CRISPR/Cas9 plasmid DNA into pronuclear. For this reason, it is expected that CRISPR/Cas9 might be used for various scientific research. The knockout alleles are generated through site-specific double strand break repair, which causes unpredictable insertions or deletions of various sizes by non-homologous end joining. To generate optional single nucleotide mutation (SNM), we co-microinjected both the CRISPR/Cas9 plasmid and the single stranded DNA donor into mouse one cell embryos. As a result, we obtained the mice bearing designed SNM alleles by homology-directed repair. We also generated the reporter knock-in allele by co-microinjection of the CRISPR/Cas9 plasmid and the double stranded DNA donor. Moreover, mutant mouse with genomic deletion greater than 1 Mb in size was generated by co-microinjection of two different CRISPR/Cas9 plasmid vectors. These results suggest that combinations of the CRISPR plasmid vector and DNA donor, and two different CRISPR plasmid vectors are able to make more flexible genome editing in mice.

P-07: CRISPR/Cas9 Gene Targeting of a Gene Refractory to Conventional Methods

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Muscle phosphofructokinase is an essential enzyme for glucose homeostasis. Patients with phosphofructokinase deficiency present with Tarui disease and exercise intolerance in the clinic. In order to generate a mouse model of this disease, exon 3 in the mouse *Pfkm* gene was selected for gene targeting in mouse embryonic stem (ES) cells. Mutation of exon 3 is expected to disrupt both isoforms of *Pfkm* by nonsense-mediated decay of mRNA encoding a premature termination codon. A gene-targeting vector designed to replace exon 3 with a sequence flanked by loxP sites was introduced into mouse ES cells. Genetic screening of 480 drug-resistant ES cell clones showed that none of them had undergone homologous recombination with the targeting vector. Subsequent efforts to generate a mutant mouse model turned to the direct manipulation of the mouse genome in fertilized eggs by the microinjection of nucleases targeted to exon 3. Four transcription activator-like effector nucleases (TALENs), one zinc finger nuclease (ZFN), and one CRISPR- associated Cas9 nuclease were designed to target *Pfkm* exon 3. Nucleases were obtained from commercial vendors and also prepared in-house from publicly available resources. Microinjection of plasmid vectors expressing TALENs targeted to exon 3 did not produce mutant mice although this method has succeeded for other genes. The microinjection of mRNA coding for TALENs also failed to generate mouse mutants, independent of the source of the TALENs reagents. The use of *ZFN* mRNA for microinjection produced multiple mouse mutants. Co-injection of *Cas9* mRNA and guide RNA produced numerous mice for analysis. Homozygous mutant mice were found to be embryonic lethal. Factors affecting the efficiency and success of nuclease-mediated gene targeting include the activity of nucleases on target sequences and toxicity of microinjection preparations.

P-08: Kallikrein 5 and kallikrein 7 double-deficient mice generated using TALENs exhibit altered epidermal barrier

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The kallikrein-related peptidases (KLKs) are a family of 15 serine proteases that create a large gene cluster on Chromosome 19 in human and Chromosome 7 in mice. Although KLKs appear to be involved in many physiological processes, their roles in vivo are still incompletely understood partially due to unavailability of suitable mouse models. *Klk5* and *Klk7* have been identified as participants in tightly regulated proteolytic pathways that are crucial for epidermal homeostasis. Although knock-out mouse models for *Klk5* and *Klk7* have been generated, they did not show obvious phenotype. Generation of a mouse model lacking both proteases by conventional strategies is impossible since both genes are located close to each other in the same locus and thus *Klk5 Klk7* (*Klk5/7*) -deficient mice were generated using microinjection of TALEN mRNA targeting *Klk7* into *Klk5*-deficient oocytes. These C57Bl/6N-*Klk5*$^{tm2a(KOMP)Wtsi}$/*Klk5*$^{tm2a(KOMP)Wtsi}$ *Klk7*^{em1Rase}/*Klk7*$^{em1Rase/}$ double deficient mice are viable but show severe phenotype in altered barrier integrity and peculiarly strong thickening of the skin.

P-09: Targeting specific DNA sequences by *N*-methylpyrrole and *N*-methylimidazole polyamides provides insights for the development of novel diagnostic and therapeutic drugs

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DNA-binding small molecules that can recognize specific sequences of genomic DNA would serve as novel tools to detect specific sites of DNA and manipulate gene expression. *N*-methylpyrrole (P) and *N*-methylimidazole (I) polyamides are synthetic DNA minor groove binders that have been shown to affect gene expression by competing with DNA-binding proteins in a sequence-specific manner. To apply the chemical compounds to the development of novel medicinal and/or diagnostic tools, we have introduced different bioactive molecules to PI polyamides. The treatment of FITC-labeled PI polyamides to cultured cells demonstrated that FITC-PI polyamides were quickly localized in the nuclei. Intravenous and intraperitoneal administration of FITC-PI polyamides into mice revealed that PI polyamides showed nuclear localization of various tissues in *in vivo* model, suggesting that PI polyamides can be used as a drug-delivery agent. To modify gene expression regulated by histone modification, we conjugated PI polyamides with histone deacetylase inhibitor, SAHA, and found that its treatment in cultured cells induced the endogenous expression of targeted genes including *Pou5f1* and *Nanog*. We also conjugated a DNA alkylating agent (seco-CBI) to PI polyamide that selectively bound to the specific DNA sequence of the mutated *KRAS* gene and alkylated mutated allele in human colon cancers with a minimum off-target damage. The treatment of alkylating PI polyamides exhibited a significant growth inhibition in colon cancer cells with a very low IC_{50} (10-50 nmol), and suppressed tumor growth exclusively in xenograft colon cancer models bearing the *KRAS* mutation with low host toxicity. Overall, PI polyamides are useful tools to develop novel strategies of diagnosis and therapy in human diseases.

P-10: The role of Large MAF Transcription Factors in Mouse Liver Reprogramming to b-like Cells

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Recent studies have shown that MAFB and MAFA have their distinctive roles in b-cell development and maturation, respectively. However, what causes the discrepancy in b- cell is not clearly explained yet. Their different gene expression timing could be one reason; *Mafb* is expressed before birth and *Mafa* after birth. Our aim is to examine the functional difference between these closely related genes in b-cells using an *in vivo* mouse model that is designed for b-like cell detection, FVB/N-Tg(Ins1-luc)VUPwrs, a MIP-luc reporter mouse. We monitored the insulin transcriptional activity using bioluminescence emitted from the liver of insulin promoter-luciferase transgenic mice upon gene transfers. Adenoviral gene transfers of *Pdx1/Neurod1/MafA* (PDA) and *Pdx1/Neurod1/Mafb* (PDB) combinations generated intense luminescence from the liver with the peak emission 3 days after transduction, which lasted more than a week. Although the signal intensities of PDA and PDB were comparable, only PDA gene transfer resulted in significant luminescence on day 10, suggesting that the role in maintaining insulin gene activation is higher in *Mafa* compared to *Mafb*. PDA gene transfer induced expression of genes that are necessary for glucose sensing and insulin secretion in the liver on day 9. However, glucose tolerance test and liver perfusion experiment showed that induced b-like cells did not respond to high glucose concentration. These results suggest that *Mafa* has a markedly intense and sustainable role on b-like cell production in comparison with *Mafb*.

P-11: Age-related differences in behavior and neuronal morphology of Diversity Outbred mice

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The Jackson Laboratory

The Diversity Outbred (DO) mouse population is a genetic mapping resource with high precision, genetic polymorphism, and phenotypic diversity. The DO provides a powerful tool for identifying genetic loci that influence behavioral and neuroanatomical changes that occur in the aging process. In a cross-sectional design we assayed 200 male and female DO mice of three age groups (~6, ~12, and ~18 months). Behavioral assays consisted of the Open Field (OF), T-Maze, Novel Object Recognition (NOR), and the Tail Suspension Test (TST). Behavioral measures are correlated with measures of spine density, spine type, and neuronal arborization from pyramidal neurons in the CA1 hippocampal subfield.

General linear models were used to test effects of age, sex, and age x sex interactions. In the OF older mice showed lower anxiety than younger mice. In the NOR we observed a significant decrease with age on distance traveled and time spent exploring objects. Time spent exploring the objects on Days 1 and 2 was correlated but was not predictive of novel object preference. Chi-square analysis showed an age effect on the proportion of poorly performing mice on novel object recognition in males but not females. No age difference was found in proportion of top performers, indicating that the proportion of older mice of both sexes displaying spared cognition is indistinguishable from younger mice. T-Maze behavior varied across age as measured by the percent of correct transitions and distance traveled. The correlation between dendritic spine density of CA1 pyramidal neurons and cognitive performance on the T-maze varied in magnitude across ages.

The behavioral results reflect age-related changes in activity, anxiety, and cognition related phenotypes. By combining these data with neurological, physiological, and genetic information obtained from the same mice we will be able to uncover genetic mechanisms and pathways of age related behavioral variation.

P-12: Generation and characterization of novel mouse models of Parkinson’s disease

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Parkinson’s disease (PD) is the second most common neurodegenerative disorder; ~1.5% of people over 65 years of age are affected (~ 1 million in the U.S.). While there are treatments that can temporarily relieve some symptoms, there is no cure available. For decades a few genetic risk factors for PD were known, but a lack of understanding of the underlying causes of PD hindered the development of new treatments. Now new genetic analysis techniques are implicating numerous genes in PD pathogenesis. Our aim is to develop animal models of PD that will be more predictive for therapy development in stratified patient populations.

One area of focus has been the creation of models with defects in pathways responsible for protein trafficking and degradation, as mutations in these pathways have been associated with PD incidence in patient populations. For example, carriers of certain mutations in the *GBA* gene are at increased risk of developing PD. Mutations in *GBA* are believed to perturb lysosomal degradation pathways, potentially leading to toxic accumulation of α-synuclein. A novel *Gba* D409V knockin mouse model, C57BL/6N-$Gba^{tm1.1Mjff}$/J, that mimics a mutation found in PD patients has been developed. Other autophagy-deficient models relevant to PD include an *Atp13a2* knockout, B6N.129S6(Cg)-$Atp13a2^{tm1Pjsch}$/J, which exhibits α-synuclein accumulation and sensorimotor deficits, and a *Vps35* D620N knockin, B6(Cg)-$Vps35^{tm1.2Mjff}$/J, which can be used to create a conditional knockout. Comprehensive characterization of molecular and phenotypic aspects in these models at 4, 8, and 12 months is in progress.

Reproducibility of preclinical studies is compromised when standard, well-characterized animal models are not available; The Jackson Laboratory PD Resource provides these models to both academic and pharmaceutical/biotechnology researchers. Updated availability and phenotypic characterization data will be presented at http://research.jax.org/grs/parkinsons.html.

This work is supported by the Michael J. Fox Foundation for Parkinson’s Research.

P-13: Cancer Susceptibility Loci and Modifier Loci: Insights from Inbred Mouse Strains

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Colorectal and small intestinal cancers afflict almost half a million people worldwide each year. The adenomatous polyposis coli (*APC*) tumor suppressor gene is mutated in more than half of all colon cancers. In addition, inherited and *de novo* mutations in the *APC* gene cause Familial Adenomatous Polyposis (FAP), a disorder that predisposes individuals to developing intestinal polyposis and eventually leads to cancer. In mouse models, the genetic background of mice carrying a mutation in the murine homolog of the *APC* gene is critical to the manifestation of tumor phenotypes, as inbred strains have been shown to differ dramatically in their susceptibility to polyposis. Carcinogen-induced tumor formation within the intestinal tract has also been shown to be dependent on inbred strain background. Complex trait analyses from several laboratories, including our own, have identified modifier and susceptibility loci that alter intestinal tumor phenotypes in mice, but only a few causative genes have been identified to date. We review the literature and report the collective results of several quantitative trait loci (QTL) studies that have exploited the genomic diversity among inbred strains to identify loci that alter tumor phenotypes. We compare the results with cancer susceptibility loci identified through Genome-Wide Association Studies (GWAS) and other studies in the human genome. The usefulness of the mouse as a model organism for optimizing complex trait screens for cancer phenotypes and speeding the process of causative gene identification is discussed. Research supported in part by NCI grants to LDS.

P-14: *R2d2* is a selfish genetic element that drives recurrent selective sweeps on mouse Chromosome 2

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Positive selection is a key driver of evolution. Strong selection of a mutation with a positive effect on fitness leaves a signature of decreased genetic variation within the region linked to the mutation (selective sweep). Most selective sweeps are caused by genes with obvious roles in recent adaptation. However, it has been proposed that “selfish” genetic elements (those that propagate without having positive effects on organismal fitness) may leave signatures identical to “classical” selective sweeps. *R2d2* is a large copy-number variant associated with non-random segregation of mouse Chromosome 2 during female meiosis. We tested ten independent breeding populations from two different outbred stocks and found that alleles of *R2d2* with high copy number ($R2d2^h$) experienced rapid increases in frequency. In the randomized Diversity Outbred (DO) population, an $R2d2^h$ allele contributed by the WSB/EiJ inbred strain underwent a more than three-fold increase (from 0.18 to 0.62) in 13 generations, accompanied by distorted allele frequencies across a ~100 Mb linked region. We also observed selective sweeps in five of eight lines (HR1-8) derived from the ICR outbred population, in which $R2d2^h$ is segregating. Three of four lines selectively bred for high wheel running and two of four control lines went to fixation in less than 60 generations. In an $R2d2^h/0$ advanced intercross (HR8xC57BL/6J), we observed that the $R2d2^h$ allele frequency increased to 0.85 in just 10 generations. Based on simulations, these changes in $R2d2^h$ frequency are occurring at least an order of magnitude more rapidly than what is possible by drift alone. To our knowledge, this is the first direct evidence of populations actively undergoing selective sweeps due to meiotic drive. Our results demonstrate that meiotic drive is a powerful evolutionary force that may rapidly alter the genomic landscape in favor of mutations with neutral or negative effect on overall fitness.

P-15: Mapping Quantitative Trait Loci with Genotyping Microarray Intensities

(See abstract SO-03 in the student/postdoc session)

P-16: InstantGenotype: A Non-parametric Model for Genotype Inference Using Microarray Probe Intensities

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Numerous microarray genotype calling methods rely on fitting a parametric model to clusters derived from the hybridization intensities of training data. However, in most cases we are uncertain about the expected sample distribution and the resulting parametric model tends to be inaccurate if the assumptions of the data distribution are not met. Moreover, most of the traditional genotype calling methods assume four genotypes (reference allele, alternate allele, heterozygous allele, or no call). The assumption gives an incomplete picture of the possible variations. Because of unexpected polymorphisms in the probe sequence and copy-number variations (including deletions), markers may capture more information than just the four expected cases.
We propose a model which makes no assumption about the data distribution and represent cluster distribution using a non-parametric model which is consistent with the data. The model can be easily evaluated and it provides genotype calls for a given sample's marker using a table lookup. Furthermore, our model has no prior assumptions concerning the number of genotype calls. We apply the algorithms to each marker separately whereas others infer a common set of clusters that apply to all probes.
We demonstrate our methods on Collaborative Cross (CC) genetic reference mice population and all samples are genotyped using a 78,000-marker genotyping array on Illumina platform. Our algorithm exhibits high concordance with Illumina genotype calls and achieves > 98% call rates on all CC samples. In addition, for a given unknown sample in the marker, our genotype-calling model is able to provide results in two ways. One can infer the posterior probabilities of the clusters for the given sample in the marker. Alternatively, it can return the genotype call with maximum probability. The web tool for the genotype-calling model is available at http://csbio.unc.edu/CCstatus/index.py?run=GenotypeInferenceMM.

P-17: Genome evolution by duplication and differential loss in *Mus musculus*

(See abstract SO-09 in the student/postdoc session)

P-18: The Y Chromosome histone demethylase KDM5D influences H3K4me3 chromatin remodeling during meiotic progression

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An important question in epigenetics is identifying the mechanisms that establish and maintain epigenetic marks. One such mark, trimethylated histone 3 lysine-4 (H3K4me3), is a prominent feature of activated chromatin, which in germ cells marks promoters, insulators, active recombination hotspots, and other sites. One of the genes potentially involved in the regulation of these marks is *Kdm5d,* a histone 3 lysine-4 demethylase on the Y Chromosome in mice and humans. To study the role of *Kdm5d* in chromatin remodeling, we developed two new transgenic mouse lines on the C57BL/6J (B6) genetic background, one with a single copy of *Kdm5d* inserted in the X Chromosome (Tg(Kdm5d)XPtkv), and another with two additional copies of *Kdm5d* on the Y Chromosome (Tg(Kdm5d)YPtkv). We were unable to obtain an autosomal insertion of *Kdm5d*. The transgenes were expressed in both female and male meiosis. To investigate the effects *Kdm5d* could have on H3K4 methylation patterns at both promoters and hotspots, we carried out a ChIP-seq for H3K4me3 and H3K4me1 in the first wave of spermatogenesis, which occurs in juvenile male mice. We compared the H3K4me3 patterns of 12-dpp spermatocytes carrying one, two, three and four copies of *Kdm5d*, and of 16-dpp spermatocytes with one copy of *Kdm5d*.

P-19: The genetic basis of morphological variation in the skull of the gray short-tailed opossum, *Monodelphis domestica*.

(See abstract SO-11 in the student/postdoc session)

P-20: Mapping metabolic traits in the Diversity Outbred mouse population: Are we there yet?

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Metabolic disorders such as obesity, diabetes and lipidopathies are complex and multifactorial, deriving from the interplay of genetic and environmental etiologies. We used a heterogeneous mouse population to model diversity in metabolic phenotypes and resolve genetic loci driving them. The Diversity Outbred mouse population (DO) was created from incipient Collaborative Cross recombinant inbred mouse strains generated from eight founder inbred strains. The DO is maintained in a randomized breeding strategy to minimize relatedness, and is currently at generation 17 of outbreeding harboring approximately 500 recombinations per mouse.

We analyzed 850 DO animals for over 100 phenotypic traits in a stepwise protocol performed on approximately 200 mice per step, coincident with production of 3 generations of DO per year. Hence, the study includes mice from generations 4-11 of outbreeding. Equal numbers of female and male mice began the study at wean age and followed a series of high-throughput noninvasive clinical assessments through 26 weeks of age. Half of the mice were fed standard chow and half were fed a high fat, high sucrose diet for the duration of the study.

An important challenge to using an outbred mouse population with greater than two founder strains has been in developing appropriate tools for correlation of genotype to phenotype. Coincident with our stepwise mouse study we have developed and refined a genetic mapping algorithm to manage 36 possible genotypes in the DO towards accurately identifying genetic loci underlying measured physiologic traits. Power analyses are now supported by empirical data to inform study sample size. We present the current analysis of quantitative trait loci (QTL) in our DO 850 study, which has resulted in the identification of 14 QTL for metabolic traits, 7 for body weight and 2 for heart rate. Of note are body weight QTL that are defined by distinct age ranges.

P-21: Mining the Mouse Genome Informatics (MGI) resource for mechanistic insights into disease etiology and therapeutic potential: genotypes, phenotypes, and models of human disease

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Large-scale forward and reverse mutagenesis projects and the pan-genomic phenotyping efforts of the International Mouse Phenotyping Consortium (IMPC) are yielding an unprecedented array of mouse resources aimed at building translational models of human disease. The MGI resource (www.informatics.jax.org) catalogs all mouse genes, genome features, and mutant alleles (available in mice or ES cells), granting free access to current, integrated biological knowledge spanning from sequence and variation to phenotype and disease model data. To this end, MGI has assigned official nomenclature to all multipurpose IKMC alleles, including recombinase-excised derivative alleles, and is primed to import phenodeviant datasets arising from high-throughput IMPC screens. MGI curates sex-specific phenotypes in the context of single gene and genomic mutations (spontaneous, induced or engineered), strains, and QTL, incorporating phenotype images and/or mutation defining schematics as available. All newly acquired allele/phenotype data are integrated with curated data from biomedical publications, individual laboratories, and legacy pilot projects to enable comparative analyses and correlative discoveries. Novel functionality introduces the ability to explicitly represent and explore key relationships among genome features that impact the functional architecture, expression, and phenotype/disease outcome associated with defined genomic regions or constructs.

Researchers can explore mouse phenotypes, alleles, and experimental disease models using a range of tools, including vocabulary browsers, a next-generation genome browser (JBrowse), and an advanced Phenotypes/Alleles Query Form with new search, filter, sort, and export functions. Enhanced query inputs include new terms from the Mammalian Phenotype Ontology, a unifying standard that evolves collaboratively to optimize genotype-phenotype annotation and data exchange with global phenotyping resources. Use of OMIM terms serves to associate phenodeviant mouse features and human gene mutations or disease syndromes. We present current content, improved mining of genotype-phenotype-disease correlations from various perspectives, and access to semantically-defined relationships associating complex mutations and the underlying altered gene/genome components. Supported by NIH grant HG000330.

P-22: A High-Fat, Low-Carbohydrate Ketogenic Diet Improves Aerobic Endurance in a Sex- and Strain-Dependent Fashion

(See abstract SO-02 in the student/postdoc session)

P-23: The spontaneous mouse mutation ebouriffe (*ebo*), associated with postnatal lethality, infertility and wavy hair, is a nonsense allele of the *Lrrc8a* (*Swell1*) gene.

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Forward genetics is a powerful approach to discover genes with pleiotropic effects in the mouse. We previously described ebouriffe (*ebo*), a mouse mutation with a hair phenotype and infertility. This autosomal recessive mutation arose spontaneously in a substrain of BALB/c and was localized to proximal Chromosome 2, between markers *D2Mit153* and *D2Mit64* (29.27-31.20 Mb), a region of homology with human Chromosome 9q34. Affected *ebo*/*ebo* mice show postnatal lethality, infertility (male and female), curly vibrissae and wavy hair (from postnatal day 7). Males have defective spermatogenesis with structural abnormalities of the acrosome that lead to abnormal head shapes and isolated flagellum (Biology of Reproduction 55: 355-363, 1996). In this report we describe *ebo* as a 2-bp frameshift deletion leading to a premature stop codon in exon 3 of the *Lrrc8a* gene. The truncated protein stops at residue 441, missing leucine-rich repeat domains LRR3 to LRR 17, but preserving the transmembrane domains. Based on the milder phenotype compared with the loss-of-function KO ($Lrrc8a^{tm1.1Geha}/Lrrc8a^{tm1.1Geha}$) model (Journal of Experimental Medicine 211: 929-942, 2014), we speculate that $Lrrc8a^{ebo}$ represents a hypomorphic allele. We will present a summary of the genetic analysis, as well as the phenotyping results from our FVB/N.Cg-$Lrrc8a^{ebo}$ (N8) congenic strain, with emphasis on the histopathology findings in testes and ovaries. As a final note, the *Lrrc8a* gene was very recently nicknamed *Swell1* and is considered to be the gene encoding for a long-sought protein involved in cell volume regulation.

P-24: The Jackson Laboratory Repository Resource: Mouse Strains for Modeling Human Disease

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The Jackson Laboratory

The Jackson Laboratory (JAX) has been serving the scientific community as a resource for mouse strains for more than seven decades, initially through individual JAX researchers and later through collaborative programs. A group of these specialized mouse distribution programs was consolidated in 2003 as the JAX Mouse Repository. The Repository consists of over 7,000 strains and encompasses many strains with applications for modeling human disease, including but not limited to traditional induced mutations (knockout targeted mutations, transgenic, chromosomal aberrations), spontaneous mutations, recombinant inbred strains, and recombinant congenic strains. Conditional, inducible, and other multi-purpose “tool strains” offer applications in a range of scientific fields. Candidate Repository strains are critically evaluated for scientific utility, uniqueness in a public repository, and predicted interest. All new Repository mouse strains are imported, rederived, cryopreserved, and offered to the scientific community once rigorous quality assurance standards have been met. Information is collected from donating investigators and the scientific literature to create strain data sheets describing the strain’s development, phenotype, maintenance, and other relevant features. The information dataset is further enhanced via collaboration with Mouse Genome Informatics (MGI). Researchers can search for a strain via the JAXMice website (http://www.jax.org). In order to make the vast number of JaxMice models more accessible, we have recently developed a new search interface that facilitates searches by disease term as well as by keyword and strain, allele, and gene name or synonym. A faceted interface allows quick access to specific strain sets, and the new search is iterative such that successive searches can filter an existing results set.

Researchers interested in depositing mice into a JAX repository can find a strain submission form at www.jax.org/donate-a-mouse.

The Jackson Laboratory Repository is supported by the NIH, The Howard Hughes Medical Institute, Michael J. Fox Foundation for Parkinson’s Research and other private charitable foundations.

P-25: Effect of Genetic Background and Intestinal Barrier Function in a Murine Model of Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) refers to a group of inflammatory conditions that manifest with chronic and relapsing inflammation of the digestive tract. Ulcerative colitis and Crohn’s disease comprise the two main IBD conditions and are currently estimated to affect up to two million Americans. Development of IBD is thought to result from inappropriate activation of immune responses due to defects in both the barrier function of the intestinal epithelium and the mucosal immune system. Clinical evidence supports that pathogenesis involves complex interactions between genetic and environmental factors. Differential susceptibility to experimentally induced intestinal inflammation has been identified among several inbred mouse strains. For example, acute colitis stimulated by dextran sulfate sodium (DSS) proceeded to chronicity in the C57BL/6 but not in the BALB/c strain. Additionally, it has been shown that genetic knockouts of the ion transporter CLCN2 have compromised barrier function and increased disease severity when exposed to DSS. Presently, we have begun to focus on the importance of *Clcn2* on distinct genetic backgrounds. Severity of disease is typically assessed by examining body weight, histopathology, and intestinal permeability. We are using these measures combined with changes in visceral hypersensitivity to generate our disease indices. Visceral hypersensitivity is quantified using colorectal distension (CRD) and may be used to detect more subtle changes in disease status. Responses to CRD are measured as electromyography (EMG) recordings of the abdominal musculature. Early efforts to detect visceral hypersensitivity included optimization of surgical techniques to properly secure electrode wires into the abdomen. The use of shortened externalized wires (~2cm) combined with suture adhesive has proved to be the most successful method for preventing dislodgement of the wires or chewing by the animals. Ultimately, we plan to identify genetic links that underlie differences in acute versus chronic inflammation and investigate the role of barrier function in IBD.

P-26: Industrial-Scale DNA-seq analysis enabling the 99 Lives Cat Genome Project

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Next generation sequencing has ushered in the era of massive data sets that require specialized bioinformatics expertise, enhanced computing resources, and ample time to extract knowledge from raw data. Here we describe a complete DNA sequencing "analysis kit" on the Maverix Analytic Platform, with robust scalability to handle massive projects. A biologist-friendly web-based interface requires only a few steps to configure and launch variant detection using industry-standard tools including GATK and FreeBayes. Key results are summarized in reports, while individual annotated variants can be explored interactively via sorting and filtering on annotated qualities. For those who wish to visualize the full genomic context of variants, a link is provided to a secure, private version of the UCSC Genome Browser for advanced exploration. We demonstrate this powerful integrated system with early successes from the 99 Lives Cat Whole Genome Sequencing Initiative, led by Professor Leslie Lyons at the University of Missouri. By studying variants in cats of different breeds and physical characteristics, consortium researchers have started to identify mutations correlated with genetic causes of feline illnesses, enabling development of new diagnostics and leads for future therapies. Numerous findings will also have implications to human diseases, such as spinal muscular atrophy. The complete analyses will be made freely available to the public as a "Community of Discovery" to maximize their value and encourage deeper exploration.

P-27: MGI: supporting mouse research; providing translational opportunities

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The mission of the Mouse Genome Informatics (MGI, http://www.informatics.jax.org) resource is to provide integrated genetic, genomic, and biological data to facilitate the study of human health and disease. To fulfill this mission, in its first 25 years, MGI has undergone dramatic growth and significant directional changes, as has the science MGI supports and the computing tools MGI uses for its infrastructure.

Today, MGI provides critical core data on mouse genes and genome features, curating their functions, expression, allelic mutations and phenotypic consequences, and associating specific allele/strain combinations to the human diseases and cancers they model. Relationships among mouse genome features and between mouse and human (and other vertebrate) genes support comparative biology. The power of MGI lies in its long-standing principle that integrating data from different sources using semantic standards maximizes the value of that data. Integration efforts in MGI thus are key at every level, from harmonizing the genome assembly builds of NCBI and Ensembl to produce a unified genome feature catalog, to developing and applying ontology tools, such as the Gene Ontology, Mouse Anatomy Ontology, and Mammalian Phenotype Ontology. With the expansion of 'big data' projects, the continued economies of sequencing, and the new technologies for genome editing, MGI will continue to evolve, remaining a hub for providing sematic standards and integrated views of mouse biological information and the close comparison between mouse and human.

Here we provide a look at the diverse sources and content of MGI data, highlighting key integration and semantic standards, and introduce the Human-Mouse: Disease Connection (HMDC) translational tool that provides new data displays for visual phenotype/disease comparison and model building. MGI components are supported by NIH grants HG000330, HD062499, CA089713, OD011190, and HG004834.

P-28: Screening of a genetically complex population leads to discovery of a novel polymorphic pathway contributing to Influenza A Virus infection.

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The importance of host genetic variation on the initial responses to, and the eventual outcomes of pathogen infections is being increasingly appreciated. The identification and characterization of the polymorphic genes and pathways that contribute to differential disease outcomes can increase our understanding of these disease processes, as well as aid in the development of therapeutics and prophylactics. The Collaborative Cross recombinant inbred mouse panel provides a population with which to assess the role of host genetic polymorphisms on multiple stages throughout viral infection. We are conducting a screen of the innate and adaptive immune responses to influenza A virus infection in a panel of F1 animals between Collaborative Cross lines (CC-recombinant inbred intercrosses, or CC-RIX). Analysis of data from this screen suggested that inbred line CC017/Unc would be highly susceptible to influenza A virus infection. We have confirmed this hypothesis through direct contrasts of CC017/Unc, C57BL/6J and the highly susceptible strain DBA/2J. Importantly, DBA/2J's increased susceptibility is thought to largely derive from a non-functional *Hc* allele and therefore a defective Complement cascade, whereas all 8 founders of the CC have functional *Hc* alleles, suggesting a novel mechanism working within CC017/Unc. Therefore, we have characterized a variety of immunological, virological, molecular and pathological responses within these three inbred lines, as well as reciprocal F1 animals between them to better understand the polymorphic response pathways contributing to a novel pathway of increased susceptibility to influenza A virus.

P-29: Modifier Gene Discovery Using a Novel ENU Mutagenesis Approach

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The discovery of modifier genes in human and mouse has seen slow progress. In mice, this is largely due to the reliance on the variability inherent in genetic background strains; identifying the causal variant even in highly resolved mapped loci has proved challenging. We have developed a fast and cost-effective method for genetic mapping using Next Generation Sequencing that combines single nucleotide polymorphism discovery, mutation localization, and potential identification of causal sequence variants. Importantly, this approach can potentially be applied to mutagenized mice that have not been outcrossed, using the ENU-induced variants as SNP markers. This will enable the possibility of doing both primary screens and modifier screens on fully inbred lines.We have begun a test of this approach using sensitized strains that cause a variety of skeletal defects, as these can be readily assessed in late gestation. We selected the following C57BL/6 congenic strains: a "knock-in" strain carrying the Pfieffer syndrome P250R mutation in *Fgfr1* (*Fgfr1$^{tm2.1Cxd}$*), a loss-of-function allele of the SHH transcriptional effector *Gli2* (*Gli2^{tm1Alj}*), a strain with loss of the osteoblast-specific isoform of *Runx2* (*Runx2^{tm1Ldq}*), and a spontaneous loss-of-function allele of *Pibf1*. The Pfeiffer model exhibits variable craniosynostosis, while the *Gli2* mutation can be modified by prenatal ethanol exposure, leading to holoprosencephaly phenotypes. The *Runx2* line could lead to identification of modifiers of bone growth and mineralization, and the *Pibf1* mutant's cleft lip, cleft palate, and ciliopathy-like phenotype could reveal modifiers of craniofacial development and function of the primary cilium. In the simplest cases, whole-genome sequencing will reveal areas of homozygosity shared between mutants with a consistent "modified" phenotype, and allow us to narrow our focus to the induced variants included in these homozygous regions. We anticipate that we may ascertain more complex genetic interactions as well.

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P-30: Genome-wide association study of behavior in an advanced intercross line of mice

(See abstract SO-05 in the student/postdoc session)

P-31: Determining the Mutifactorial Basis of Sudden Unexpected Death in Epilepsy

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Sudden unexplained death in epilepsy (SUDEP) is associated with seizures and can occur at any age without warning. While certain mutations in neural and cardiac ion channel genes have been associated with SUDEP risk, these genes do not predict risk for the majority of the epilepsy population. Clinical investigations have identified abnormal brain, cardiovascular and autonomic responses in SUDEP cases indicating that multiple mechanisms can contribute to SUDEP in mammals.

To date, no human genome wide association studies for SUDEP have been performed due to the complex nature of the disease. Alternatively, genetic models like the Hybrid Mouse Diversity Panel (HMDP) can be evaluated for SUDEP with greater control over environmental and genetic interactions. We have tested over 75 inbred lines to date using the repeated-flurothyl model of epileptogenesis in the HDMP and have observed that, similar to humans, mice likely have multifactorial responses to Seizure Induced Sudden Death (SISD). Our work has identified Quantitative Trait Loci (QTL) linked to seizure severity and progression as well as seizure related death. Identification of novel gene variants in our preclinical model will provide mechanistic insight into the pathophysiology of SUDEP.

We hypothesize that genetic factors, combined with environmental stressors, give rise to distinct subtypes of SISD and that identification of these modifiers will reveal novel SUDEP risk factors for testing in humans. Our ongoing work will expand our SISD model to identify the molecular and physiological basis of SUDEP in the HMDP though multidisciplinary efforts to comprehensively determine the causes of death and their association with specific modifiers.

P-32: Integrating SNPs, epigenetics and transcriptomics to better understand the inherited predisposition to breast cancer metastasis

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Breast cancer is the most frequent cancer and the second leading cause of cancer mortality in women. The vast majority of breast cancer mortality is due to metastatic disease since the primary tumor can be relatively easily surgically resected. More comprehensive understanding of biology of metastasis is therefore clearly warranted to unveil novel metastasis-associated molecules and cellular processes that might be targeted for clinical intervention. Previously we demonstrated that like cancer incidence, metastatic progression has a significant inherited component. Using mouse complex trait mapping strategies we have been isolating metastasis susceptibility genes from backcross analysis. However, studies suggest that many of these QTL peaks are the result of the contribution of multiple genes, suggesting that causal genes are not being identified. To increase our ability to detect causal genes we have implemented a new integrated strategy. Whole genome sequencing of appropriate high or low metastatic mouse strains is performed to identify variants, which are then filtered for those within DNAse hypersensitive sites (DHS), based on the hypothesis that most inherited phenotypes are due to expression differences rather than missense variants. The genes associated with polymorphic DHS are then screened through mouse and human tumor expression databases to identify those genes associated with development of metastatic disease. This strategy was able to identify genes previously associated with metastatic breast cancer in both mouse and humans and new genes have also been validated. The results further suggest that inherited variation in cellular mediated immune response may be an important contributor to metastatic disease.

P-33: Network Detection for Susceptibility Genes Predisposing to Intestinal and Colon Cancers

(See abstract SO-06 in the student/postdoc session)

P-34: Identifying Key Driver Events in the C3H- *Chaos3* Mouse Model for Breast Cancer

(See abstract SO-07 in the student/postdoc session)

P-35: Variability in empathic fear among 10 inbred strains of mice

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Empathy is an important emotional process that involves the ability to recognize and share emotions with others. We have previously developed a simple behavioral assay to measure fear empathy in mice. In observational fear learning (OFL), mice learn fear without receiving direct adverse stimuli when they observe a conspecific demonstrator receiving repetitive foot shocks. Although we have previously identified that the calcium channel, voltage-dependent, L type, alpha 1C subunit, *Cacna1c*, (Cav1.2 calcium channel) in anterior cingulate cortex (ACC) plays an important role in modulation of OFL, genetic factors regulating empathy are largely unknown. By comparing 10 commonly used inbred mouse strains, we found that fear empathy was highly variable and strain dependent. Four strains-C57BL/6J, C57BL/6N, BTBR T^{+} $Itpr3^{tf}$/J, and 129S4/SvJae showed significant levels of freezing in OFL, whereas AKR/J, BALB/cByJ, C3H/HeJ, DBA/2J, and FVB/NJ, and NOD/ShiLtJ exhibited impaired empathy. To further characterize the differential empathic response between these strains, we conducted a set of behavioral task to examine fear conditioning, anxiety, locomotor activity, and sociability. Most strains that exhibited impaired empathy also showed low levels of freezing in a conventional fear conditioning task. However, despite similar levels of freezing to that of C57BL/6J strain in fear conditioning, strain AKR/J, BALB/cByJ, and DBA/2J mice exhibited impaired empathy in OFL task. Anxiety, locomotor activity, or sociability were not correlated with the differential fear empathy between the 10 strains. Taken together, these data strongly suggest that a genetic variation(s) in genes modulating empathy in mice. The identification of causal genes may uncover novel genetic pathways and underlying neural circuits that modulate empathy and, ultimately, provide new targets for therapeutic intervention in human mental disorders.

P-36: Analysis of the host response to influenza A virus infection in the Collaborative Cross founder strain

(See abstract SO-08 in the student/postdoc session)

P-37: Collateral damage: Identification and characterisation of spontaneous mutations from a targeted knockout programme

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Making targeted mutant alleles involves extensive manipulation of ES cells, and the resulting knockout cell lines could potentially carry multiple spontaneous mutations as well as the intended targeted mutation. So far, twenty lines from the Sanger Institute's Mouse Genetics Project have been identified as carrying a spontaneous mutation affecting hearing. These were all discovered during routine phenotyping; the hearing test used, the Auditory Brainstem Response, is a very sensitive test and can detect subtle hearing defects. Of the many affected lines, six distinct phenotypes have been observed, and seven lines have been isolated representing four of those phenotypes; early severe progressive hearing loss, later onset progressive hearing loss, low frequency progressive hearing loss and complete deafness with vestibular dysfunction.

Using a combination of linkage analysis, exome sequencing, complementation tests and expression analyses, several of the genes involved in these phenotypes have been identified, including both new alleles of genes known to be involved in hearing, and new genes not previously associated with deafness. Interestingly, there is no indication of large alterations to the genome; thus far all the identified mutations have been small, for example, single base pair missense mutations. In addition, not all the lines grouped under a single phenotype have proven to have the same mutation.

ES cell manipulation is a commonly used technique, and the creation and characterisation of mutant mice is critically important for exploring gene function. This study highlights the unintended consequences of ES cell manipulation, which in this case have proven to be an unexpected benefit. Further analysis will not only offer insight into the mechanisms of hearing loss but also into the mutations which can result from large-scale ES cell manipulation.

P-38: Integrated analysis of the epigenetic landscape for immortalized melanocytes switching from a proliferative to HIF1A induced cellular state

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Melanocytes function to produce melanin pigment, which is responsible for the diverse hair coloration patterns in mammals and for protection of human skin cells from ultraviolet radiation exposure. Gene expression in melanocytes can vary greatly depending on the stage of development and the melanocyte position in the body axis, in both cases responding to distinct signals present in the surrounding tissues. Our work is focused on understanding cellular cues that allow the melanocyte to regulate proliferative vs. migratory cell fate changes, both during development and during melanoma tumor growth and metastasis. We have established cis-regulatory genomic region dataset using Melan-a (*Cdkn2a* -/-) immortalized mouse melanocytes. This dataset includes genome wide: DNase I HS regions; enhancer regions marked by H3K4me1 and EP300 co-binding; ChIP-Seq for SOX10, TFAP2A and HIF1A transcription factors; and RNA-SEQ datasets under proliferative and HIF1A-activated growth conditions. Using this dataset, we are applying an integrated functional genomics approach to identify how the cis-regulatory sequences are utilized to regulate gene expression, identify the key factors that are coordinately engaged at these sites, and assess the role that DNA variation has in modulating factor binding and gene transcription in the switch from proliferative vs. hypoxic cell signaling conditions. By identifying the epigenetic cis-regulatory interactions that occur as a result of HIF1A activation, we hypothesize that we will highlight pathways important for melanoma progression that may be relevant to developing therapeutic approaches.

P-39: An Allelic Variant of *Mtor* Associated with DNA Damage Response and Tumor Susceptibility

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Mouse plasma cell tumors share several pathogenetic features in common with human Burkitt's lymphoma and multiple myeloma. The genetics of plasmacytomagenesis has been shown to be a complex trait and one of the susceptibility genes implicated is the mammalian target of rapamycin, *Mtor*. During the course of these studies, we generated a knock-in (KI) mouse carrying the BALB/c allele $Mtor^{tm1.1Lgm}$/ $Mtor^{tm1.1Lgm}$ ($Mtor^{628C}$) of *Mtor* associated with plasma cell tumor development. Gene expression profiling studies uncovered several alterations in the mTOR pathway associated with the knock-in allele and identified DNA replication, recombination and repair as one of the top pathways affected. In order to study the DNA damage response (DDR) in our KI and wild-type (WT) littermates, we performed total body irradiation experiments using a single 8 Gy dose of gamma radiation. Survival was followed over the course of 30 days and the KI mice carrying the $Mtor^{tm1.1Lgm}$/ $Mtor^{tm1.1Lgm}$ had a lower survival rate than either their wild-type or heterozygous littermates. Radiation dose responses and time courses are being performed to uncover the DDR phenotypes in WT and KI mouse embryonic fibroblasts (MEFs) with respect to genes and proteins in the *Mtor* pathway.

P-40: Mapping of QTL modifying *Hbb-bh1* expression in *Klf1*^{Nan} F2 and Diversity Outbred (DO) mice

(See abstract SO-10 in the student/postdoc session)

P-41: The genetic basis for susceptibility to the acute hepatitis form of Rift Valley fever disease in MBT/Pas inbred mice

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Rift Valley fever virus (RVFV) can cause severe disease in livestock and humans. Variable clinical manifestations of the disease in livestock and humans suggest a role of genetic determinants influencing the progression and severity of the disease. These genetic factors are still unknown. The systemic inoculation of mice with RVFV reproduces major pathological features of severe human disease, notably the rapid onset hepatitis and delayed onset encephalitis. Here, we use quantitative trait locus (QTL) linkage mapping and functional analysis in congenic strains to demonstrate that three QTLs regulate the susceptibility of MBT/Pas inbred mice to RVFV infection. Two sex-specific QTLs were mapped on Chromosomes 2 and 5. A genetic interval *Rvfs2* on Chromosome 11 reduces the survival of RVFV-infected BALB/cByJ mice in both sexes. Moreover, the *Rvfs2* locus controls the RNA viral load in the peripheral blood, and the severity of the pathology in the liver. *Rvfs2* protects against the deleterious outcomes of early acute hepatitis, but not against those of the late onset encephalitis. This first genetic investigation of susceptibility to RVFV infection in mice indicates that host genetic elements influence the development of different forms of the disease in either females or males or across both sexes.

P-42: Mouse Models of Skin, Hair, and Nail Diseases in The Jackson Laboratory Mouse Mutant Resource

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The Mouse Mutant Resource (MMR) has been in existence for over 50 years with continuous support by the National Center for Research Resources (NCRR). Mice carrying spontaneous mutations manifesting in skin, hair, and nail phenotypic deviants have been a rich resource for productive dermatologic research since the early 1900s. Despite the capability in recent decades to genetically engineer mouse models, strains harboring spontaneous mutations continue to provide the raw material for in-depth investigator-initiated research, broad phenotypic insight, nucleotide-level functional annotation of the mouse genome, large-scale genetic pathway analysis, and faithful modeling of human genetic disorders. The hundreds of thousands of mice on the JAX campus on any given day, and the millions of mice produced over the course of a year, offer a unique and powerful opportunity for the biomedical community to access spontaneous mutation mouse models of human skin disorders. Within such large populations, spontaneous mutations inevitably occur at a rate not fully appreciated in smaller colonies. Through a combination of high colony size, large diversity of inbred strains and a systematic selection for phenotypic deviants, over time, large numbers of mutations affecting many organ systems are found. These spontaneous mutations are manifested in mice (known as phenotypic deviants) with rare, unusual and biomedically relevant phenotypes. A wide variety of types of mutations occur naturally, more similar to those seen in humans, than can be produced by genetic engineering, therefore many of these are more accurate models of specific human diseases. Examples of many of the commonly used models will be presented.

P-43: Using human GWAS to mine phenotypic variation in the Diversity Outbred mouse population

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A large study of more than 100 metabolic traits in the Diversity Outbred (DO) mouse population fed standard chow or a high fat diet revealed quantitative trait loci (QTL) for hematologic and cholesterol parameters. Among 550 mice in the study, there was broad variation in body fat percent, glucose homeostasis and other traits associated with the Metabolic Syndrome. To our surprise, no significant QTL were identified for these traits using analyses designed for use in the DO, although numerous “case studies” were observed in which coincident metabolic abnormalities, including obesity, presented in individual mice. To further investigate the genetic loci underlying these traits we referred to human GWAS data to evaluate whether genes found at syntenic regions in the DO also drive metabolic traits in mice.

Human SNPs significantly associated with metabolic disorder traits were selected from the Human GWAS catalog. SNPs were filtered to only include those within or near protein coding genes, and 678 orthologous mouse genes were then identified by use of the ENSEMBL API. Because the DO derive from eight founder inbred strains, there are 36 possible genotypes at any given locus. Mice were divided into obese (OB) and non-obese (Non-OB) groups based on percent body fat. Genotypes at each locus were determined and an additive genotype associative test using a one-way ANOVA was performed for each of 100+ traits by each SNP per gene. An R script was developed to automate this process. We found significant associations of 25 metabolic traits with 1-50 genes per trait, thereby greatly enhancing the utility of our DO data to validate and further define the contribution of these genetic variants to metabolic disorders.

P-44: Exploring differential splicing and phenotypes in mouse models of human disease

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MRC Harwell conducts two large scale mutagenesis screens namely, The International Mouse Phenotyping Consortium (IMPC) which aims to find a phenotype for every gene in the mammalian genome and an ENU Aging mutagenesis screen to study the genetics of ageing. These screens provide a rich source of functional data, which can generate hypothesis about the mutation in question. In addition we use DNA-Seq to identify the ENU mutations, which lie within genes, and RNA-Seq to identify differentially expressed genes in the mutants.

The emergence of next generation RNA sequencing has provided an exciting new technology to analyse alternative splicing on a large scale. However, computational methods for analyzing differential expression and differential splicing from short read sequencing are not fully established yet and there are still no standard solutions available for a variety of data analysis tasks. One of the major challenges with RNA-Seq analysis is the identification of differential isoforms and splicing events. Isoforms and abhorrent splicing events have previously been implicated in a number of different diseases and associated with different cancer types. Therefore effective detection of isoforms and splicing events in mouse models of disease is critical to identify novel functional roles of genes relating to the phenotype.

Recently, we have sequenced the transcriptome to analyse the gene expression levels between affected and unaffected mice. Here we present our analysis on identifying differential splicing and isoform expression in ENU mutants from RNA-seq data by integrating multiple statistical algorithms. In addition we will show how regulatory networks and mechanisms contribute to the phenotype by predicting co-expressed molecular complexes in pathways. Overall we will show how differential splicing events and isoforms can contribute to abhorrent phenotypes.

P-45: Genetic Analysis of NOD Substrain Divergence

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The NOD mouse represents a premier animal model for the study of spontaneous insulitis and autoimmune Type 1 diabetes. This inbred strain was developed at Shionogi Research Laboratories (Japan) and several substrains are maintained in Japan, United States and Germany.

While the substrains were separated for less than 35 years, and the number of DNA polymorphisms is therefore limited, striking differences in diabetes penetrance have been observed. To exclude possible confounding of environmental factors like pathogens and the microbiome, two substrains were compared at the same laboratory, Diabetes Research Institute, Dusseldorf. For both sexes the significant differences were attained both in rate of diabetes onset and the total incidences.

To reveal genetic causes of the phenotypic differences, we genotyped five of NOD substrains: NOD/ShiJcl (CLEA Japan, Inc.), NOD/ShiLtJ and NOD/ShiLtDvs (The Jackson Laboratory, US), NOD/MrkTac and NOD/BomTac (Taconic Europe). Using Exome Capture Sequencing we identified 62 SNPs and 2 short indels between substrains having coding consequences. Moreover, Mouse Diversity Array genotyping uncovers deletion of several exons of *Icam2* gene in NOD/ShiLtDvs substrain and >100kb deletion on Chr 3 shared by NOD/ShiLtDvs and NOD/ShiLtJ.

P-46: High-Resolution Genetic Mapping of Atherosclerosis in the Diversity Outbred Mouse Population

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Inbred mice which exhibit strain-specific variation in susceptibility to atherosclerosis and dyslipidemia have been used to model the genetic architecture of these complex diseases. Traditional quantitative trait locus (QTL) mapping studies using inbred strains often identify large genomic regions, containing many genes, due to limited recombination. This hampers candidate gene identification and translation of these results into possible risk predictors and therapeutic targets. An alternative approach is the use of multi-parental outbred strains for genetic mapping, such as the Diversity Outbred (DO) mouse panel. We fed 292 female DO mice either a high fat, cholesterol-containing diet to induce atherosclerosis or a non-atherogenic, low-fat, high-protein diet for 18 weeks and measured plasma lipid levels before and after diet treatment. We measured markers of atherosclerosis in the mice fed the high fat, cholesterol-containing diet after diet treatment. The mice were genotyped on a medium density array and founder haplotypes were reconstructed using a hidden Markov model. The reconstructed haplotypes were then used to perform linkage mapping of atherosclerotic lesion size as well as plasma total cholesterol, triglycerides, insulin, and glucose. Among our highly significant hits, we detected a ~100 kb QTL interval for atherosclerosis on Chromosome 6 in the mice fed an atherogenic diet, as well as a 1.4 Mb QTL interval on Chromosome 9 for triglyceride levels at baseline and a coincident 22.2 Mb QTL interval on Chromosome 9 for total cholesterol after dietary treatment. One candidate gene within the Chromosome 6 peak region associated with atherosclerosis is *Apobec1*, the apolipoprotein B mRNA editing enzyme, which plays a role in the regulation of APOB, a critical component of LDL, by editing *Apob* mRNA. This study demonstrates the value of the DO population to improve mapping resolution and aid in the identification of potential therapeutic targets for cardiovascular disease.

P-47: Refinement and alignment: Combined genetic mapping and whole locus sequencing for the granulosa cell tumor susceptibility 1 (*Gct1*) locus in SWR mice

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Introduction: Female mice of the SWR/Bm (SWR; allele *SWR*) inbred strain are susceptible to the spontaneous initiation of juvenile-onset granulosa cell (GC) tumors of the ovary at puberty. Forward genetic mapping strategies have confirmed strong linkage between GC tumor susceptibility and a locus on distal Chr 4 named granulosa cell tumor susceptibility 1 ($Gct1^{SWR}$). Phenotype-driven mapping using subcongenic lines incorporating genome from the tumor-resistant *castaneus* (CAST/Ei) strain has narrowed the *Gct1* interval to 1.31 Mb and 17 annotated genes. We hypothesize that $Gct1^{SWR}$ is a regulatory genetic polymorphism that supports an increased probability of GC tumor initiation following normal endocrinological stimulation of the maturing mouse ovary.

Methods: Whole locus targeted capture and Next Generation sequencing technologies were combined to identify *Gct1* polymorphisms unique to the SWR strain. Common polymorphisms were eliminated by *in silico* comparison to genome sequences from tumor-resistant mouse strains. Of over 2,000 predicted novel variants, a subset was prioritized for independent verification by Sanger sequencing.

Results: Confirmed regulatory variants in four genes – *Dhrs3*, *Tnfrsf1b*, *Tnfrsf8*, and *Vps13d* – were prioritized for further investigation of their impact on gene expression and protein function. A significant variant under investigation is a putative splice site mutation in the *Dhrs3* gene*,* a short-chain dehydrogenase/reductase involved in retinoic acid metabolism.

Conclusions: The combined strategies of forward mapping and high throughput sequencing have prioritized regulatory variants in the $Gct1^{SWR}$ locus that are relevant to endocrine-sensitive GC tumor initiation in pubertal SWR female mice. Our goal is to identify pathogenic *Gct1* alleles and signaling pathway aberrations for future translation to human, juvenile-onset GC tumors.

P-48: Mapping host-pathogen genetic interactions to understand TB pathogenesis

(See abstract SO-15 in the student/postdoc session)

P-49: CHD7 and SOX11 Contributions to Inner Ear and Craniofacial Development

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Tight regulation of gene expression is dependent on interactions of transcription and chromatin architecture. CHD7, a chromatin remodeling protein, is required for proper development of tissues affected in CHARGE syndrome, a multiple anomaly condition associated with craniofacial dysmorphisms and inner ear dysfunction. While mutations in *CHD7* occur in the majority of individuals with CHARGE, other genetic lesion(s) in the remaining patients have not been well defined. We identified a 12-year-old girl who presented with bilateral ocular colobomata, short stature, developmental delay, genital dysplasia, overfolded helices, bilateral inner ear dysplasia, and severe right-sided sensorineural hearing loss. *CHD7* sequencing, duplication and deletion studies were negative. A *de novo* 6.5 Mb gain of genomic material at 2p25.3-p25.2 was detected by chromosomal microarray and confirmed by FISH. The duplicated region contained 22 genes including the SRY-related HMG box-containing transcription factor gene *SOX11.* Copy number and coding sequence analysis of 28 other *CHD7* mutation-negative patients was unrevealing. Further, recent evidence in murine neural stem cells suggests that CHD7 complexes with SOX2 and is necessary for proper expression of *Sox11* and its functionally related family member *Sox4.* Based on these observations, we hypothesized that CHD7 and SOX11 may cooperate to promote proper development of multiple CHARGE-related tissues. Interestingly, SOX11 co-localized with CHD7 in several of these tissues in the embryonic mouse, including the forebrain, cerebellum, eye, inner ear, and nose. Further, qPCR revealed a reduction in *Sox11* mRNA in the E10.5 $Chd7^{Gt(S20\text{-}7E1)Sor}/Chd7^{Gt(S20\text{-}7E1)Sor}$ ($Chd7^{Gt/Gt}$, null) otocyst. We are continuing studies of *Sox11* haploinsufficient mice and developing zebrafish and mouse models of *Sox11* overexpression. Additionally, we are conducting experiments aimed at uncovering molecular relationships between CHD7 and SOX11, which may be tissue- and developmental-stage specific. These studies will help clarify roles for SOX11 in embryologic development and pathogenesis of CHARGE syndrome.

P-50: Spontaneous 8bp deletion in *Nbeal2* recapitulates the grey platelet syndrome in mice

(See abstract SO-16 in the student/postdoc session)

P-51: Human-Mouse: Disease Connection, a tool for exploring human diseases and mouse models

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Mice are excellent animal models for studying human diseases because mice have many physiological and genetic similarities to humans and can be utilized to identify and test new therapeutics. The Mouse Genome Informatics resource (MGI, www.informatics.jax.org) has a new translational tool, the Human-Mouse: Disease Connection (HMDC) (www.diseasemodel.org), that allows researchers to access integrated mouse and human genomic, phenotypic and disease information.

The HMDC portal can be used to identify experimental mouse models of human disease based on OMIM disease associations, identify mouse genes involved in phenotypes of interest, and identify candidate genes for human disease based on phenotypic similarities between characterized mouse mutants and humans. Additionally, candidate variants from whole genome sequencing and candidates within a genomic region can be prioritized based on reported phenotypes and disease associations. Links also are provided from model data to IMSR (www.findmice.org) for obtaining mouse resources.

Investigators can search HMDC by mouse or human genes, genome locations/regions, human disease names and/or mouse phenotypes. Or users can submit VCF files for analysis. Search results display in an interactive grid enabling visual comparison of gene, phenotype and disease data. Grid cells are color-coded to distinguish mouse or human data and the depth of annotation. Users can filter grid rows/columns to choose specific genes, phenotypes, and diseases they want to compare. Grid cells are dynamically linked to underlying data detail. Results also can be viewed as gene- or disease-centric tables. For example, searching by “Alport Syndrome” reveals five mouse and seven human genes implicated in the diseases and the phenotypic information for mouse mutants modeling the syndromes (as of July 2014).

We will demonstrate the powerful and easy access to integrated data in the HMDC and illustrate how it can be effectively used to identify candidate genes and locate useful mouse models.

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P-52: Partial haploinsufficiency of the mouse *Prdm9* gene

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The PRDM9 protein participates in the determination of the sites of double-strand DNA breaks (DSBs) during meiosis. The formation of DSBs is necessary for fertility. *Prdm9*-deficient laboratory mice are sterile and their sterility is thought to be a consequence of the DSBs relocation into functional DNA elements. Testes weight and sperm count are similar in wild-type males and males heterozygous for the *Prdm9* mutation. Here we show that males heterozygous for two *Prdm9* mutations on multiple backgrounds display a mild meiotic arrest, suggesting a partial haploinsufficiency of the mouse *Prdm9* gene. The decrease in meiotic efficiency in *Prdm9* heterozygotes contrasts with the situation in some mouse intersubspecific F1 hybrids, where the deletion of the *Prdm9* gene allele comming from the laboratory mice leads to increased meiotic progress and fertility, probably due to reduced number of hybrid incompatibilities. The partial haploinsufficiency of *Prdm9* on intrasubspecific background is of medical interest, as it supports a previous finding that men carrying just one *PRDM9* mutation can be infertile.

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P-53: A Genetic Interaction Network Model of a Complex Neurological Disease

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Absence epilepsy (AE) is a complex, heritable disease characterized by a brief disruption of normal behavior and accompanying spike wave discharges (SWD) on the electroencephalogram. Only a handful of genes has been definitively associated with AE in humans and rodent models. Most studies suggest that genetic interactions play a large role in the etiology and severity of AE, but mapping and understanding their architecture remains a challenge, requiring new computational approaches. We use Combined Analysis of Pleiotropy and Epistasis (CAPE) to detect and interpret genetic interactions in a meta-population derived from three C3HeB/FeJ (C3H) x C57BL/6J (B6J) strain crosses, each of which is fixed for a different SWD-causing mutation. Although each mutation causes SWD through a different molecular mechanism, the phenotypes caused by each mutation are exacerbated on the C3H genetic background compared with B6, suggesting common modifiers. By combining information across two phenotypic measures – SWD duration and frequency – CAPE revealed a large, directed genetic network consisting of suppressive and enhancing interactions between loci on 10 chromosomes. These results illustrate the power of CAPE in identifying novel modifier loci and interactions in a complex neurological disease, towards a more comprehensive view of its underlying genetic architecture.

P-54: The Mouse Tumor Biology Database Patient Derived Xenograft web portal: *in vivo* models of human cancer

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Mouse models of human cancer are widely used to study basic biological processes in cancer and to predict responses to cancer treatment in humans. Mice that have been genetically engineered to carry specific mutations in oncogenes and tumor suppressor genes have led to insights into the genetic and molecular basis of tumor initiation and progression. However, the treatment responses of mouse tumors do not always mirror those of equivalent tumors in humans. For clinical applications, human tumors engrafted into transplant-compliant recipient mice (Patient Derived Xenografts or PDX) have an advantage over tumors that arise in genetically engineered mice because xenografts allow researchers to directly study human cells and tissues *in vivo*. The development of xenograft resources that include models created using tumors from patients with common cancer diagnoses and/or common genome properties are emerging as platforms for "virtual clinical trials" to test standard of care and experimental therapies on a large scale and with high throughput.

The Mouse Tumor Biology database (MTB) has implemented a new "PDX portal" to provide access to information and data associated with PDX models of human cancer. Currently the PDX data accessible via MTB is from The Jackson Laboratory's diverse PDX Resource developed by engraftment of human tumor tissue in the NOD.Cg-$Prkdc^{scid}$ $Il2rg^{tm1Wjl}$/SzJ (aka, NSG) mouse. MTB's PDX Search Form allows users to identify models based on a variety of parameters including organ site, diagnosis, and genome properties of the engrafted tumor. Each PDX model has a summary page to provide rapid access to information regarding somatic mutations, copy number variants, gene expression, histopathology, growth rate, and/or drug response. We have implemented data visualization tools that support graphical comparisons of data associated with genes and clinical annotations and interactive circos plots of genomic data associated with PDX models. Supported by NCI grant CA089713.

P-55: Integrating High Throughput Phenotyping Data with Curated Phenotypes in Mouse Genome Informatics (MGI)

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The International Mouse Phenotyping Consortium (IMPC) coordinates a global effort aimed at high throughput phenotyping knockout mice from the genome-wide targeted mutations of the International Knock-out Mouse Consortium (IKMC). Together with the Mouse Genetics Project of the Wellcome Trust Sanger Institute (WTSI), this systematic, broad-based primary phenotyping is producing an encyclopedic resource of gene function in mammals. Maximum biomedical value of these mutant mice will emerge from placing primary high throughput phenotyping data in context with secondary, comprehensive phenotypic analyses combined with published phenotype details on these and related mutants. Mouse Genome Informatics (MGI) has traditionally integrated published mouse phenotypes with mouse genome and functional data. MGI now incorporates high-throughput phenotyping data into this extended biological context, and uniquely integrates these data with a wealth of literature curated phenotypes. Phenotype searches in MGI access all of these data, so users can expect comprehensive result sets when combining phenotype searches with other biological information of interest (gene/gene product, genome location, gene function, disease association). Users can also compare phenotype calls from different high throughput phenotyping centers side-by-side with literature-curated analyses on the same mutants, revealing shared and differing interpretations, and can easily compare phenotypes of these mutants with different genetic backgrounds or if generated from different ES cell clones. Access to primary data is provided via links to the IMPC Portal and to primary publications. In addition, MGI's Human-Mouse Disease Connection provides more clinical-friendly access to these high (and low) throughput mouse phenotypes, with associated human/mouse genes and disease associations. We present views of integrated phenotypic data in MGI, and show how these contribute to the great value of ongoing genome wide phenotyping efforts. We also discuss future plans to place these phenotype annotations into an improved genome context.

Supported by NIH grant HG000330

P-56: Spontaneous Insights from The Mouse Mutant Resource: Hypermorphs, Hypomorphs, and Novel Gene Mutations

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For over 50 years the Mouse Mutant Resource (MMR) has characterized spontaneous mouse mutants and more recently has performed the forward genetic analysis to determine the underlying molecular mutations. As the Knockout Mouse Project endeavors to create and characterize targeted knockouts of all expressed genes, these spontaneous mutants persist in providing unique models for human disease. This is in part due to the inherent requirement for viability present in our phenotype screen and the fact that the large volume of mice bred at The Jackson Laboratory every year provides a rich source of novel mutations, many of which are identified as part of our genetic quality control process. Hypomorphs and hypermorphs can broaden our understanding of gene function beyond that learned by gene ablation, and we present several spontaneous mouse mutants that have a phenotype distinct from that of knockouts of the same gene. This understanding is valuable for predicting the impact of particular mutations on phenotypic outcome. We also present several spontaneous mutations in genes for which there are not yet any other living mouse mutants characterized, and several spontaneous mutants that have not yet had their underlying molecular mutations defined.

P-57: Mouse Phenome Database

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The Mouse Phenome Database (MPD; phenome.jax.org) is a widely used online resource providing access to primary experimental data and protocols in the predominant genetic model organism, the laboratory mouse. The MPD, in existence for the past 13 years, amasses, annotates, integrates and maintains primary quantitative phenotype data and protocols in a centralized public database. Since the inception of MPD, a wealth of phenotype technologies and mouse resources have led to an expanded scope and refocus of the system – from inbred strain characteristics to a rigorously curated data resource for complex trait and integrative genetic analysis. This resource houses phenotypic, gene expression or genotype data for >1300 strains. MPD provides a catalog of phenotypic assays, analysis tools to explore genetic variation, and a common framework for data access and data dissemination. Data come from investigators around the world and represent a broad scope of behavioral endpoints and disease-related characteristics in naïve mice and those exposed to drugs, environmental agents or other treatments. MPD provides an important venue for compliance with data sharing policies and facilitates data reuse, saving time and resources while reducing animal use. Integrating various sources of phenotype data in MPD provides researchers with the resources they need to reproduce experiments, reanalyze genetic studies with new algorithms and genetics maps, understand relationships among traits and elucidate the shared genetics for a multitude of traits. The high level of documentation and curation standards and stability of the program at The Jackson Laboratory have made MPD a primary resource for investigators to archive and retrieve quantitative mouse phenotypic data. This high-quality, standardized data resource enables investigators to select mouse strains for modeling disease, compare results of diverse phenotypic assays and benchmark experimental data using detailed protocols. Updates to the MPD system will be highlighted.

P-58: Integrative Functional Genomic Analysis using GeneWeaver.

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The functional genomics data landscape is replete with diverse, distributed and often non-computable data sets, many of which can be represented as sets of genes experimentally associated with biological concepts. Aggregation and integration of these data resources enables the development of convergent evidence in gene to trait associations and for the discovery of a shared role for genes in multiple biological functions. GeneWeaver.org is a website for performing integrative functional genomics analysis in real time. The website couples a growing database of 60,000 public curated gene sets together with analysis tools based upon graph algorithms and combinatorics which allows users to compare sets of genes across species and across experimental platform. Among the genesets include those uploaded from other community sources such as the Comparative Toxicogenomic Database, GeneNetwork and the Neuroscience Information Framework. The database can be searched by free text, retrieving results of various data types from eight species. The gene sets are collected into projects that can be analyzed alone or together with primary data that the user has uploaded. This integrative approach allows identification of new associations among genes, traits and disease. GeneWeaver is a flexible system with a very broad scope. Question can be asked such as "What genes lie at the intersection of two comorbid conditions, and which disorders share a biological basis", "Which genes are highly associated with aging and neurological decline", "What chemical substances interact with genes known to be associated with genes differentially associated with Crohn's disease". Several successful validation studies have been performed using the GeneWeaver system, including a joint candidate for alcohol preference and withdrawal, a multi-species alcohol response gene, and a second gene associated with alcohol preference and seizure. These studies reveal the value of convergent data integration to identify novel genes and mechanisms. Supported by NIH AA18776

P-59: FANTOM5, a comprehensive promoter-based gene expression atlas

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We have developed cap-analysis gene expression (CAGE) to simultaneously map mRNAs and non-coding RNAs transcription starting sites (TSSs) and measure their expression at each different promoters. Since CAGE shows single nucleotide resolution, we can use this technology to comprehensively measure gene expression at each TSSs. Due to this unprecedented resolution, we have learned that promoters use different regulatory elements in different cells and tissues. Using CAGE, we can also infer the transcriptional networks that regulate gene expression in each different cell type. For its high resolution to map TSSs, CAGE has been used extensively in the ENCODE and modENCODE projects.

In the FANTOM5 project, we have applied CAGE on a comprehensive panel of human and mouse primary cells and other tissues, resulting in a very broad map the promoterome and regulatory networks. Our map reveals the existence of more than 180,000 promoters and 45,000 enhancers, which are often tissue specific. Additionally, we have determined the pattern of expression of retrotransposon elements (RE), which are likely to have a regulatory role. As example, some families of LTR retrotransposon elements are specifically expressed in ES and iPS cells, where they have a role in maintenance of pluripotency.

The FANTOM5 database is one of the broadest expression database available to the community (http://fantom.gsc.riken.jp/5/).

P-60: Methodology for the Inference of Gene Function from Phenotype Data

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Biomedical ontologies are increasingly instrumental in the advancement of biological research. Their utility is primarily through their use to efficiently consolidate large amounts of data into structured, accessible sets. However, ontology development and usage can be hampered by the segregation of knowledge by domain that occurs due to independent development and use of the ontologies. The ability to infer data associated with one ontology to data associated with another ontology would prove useful in expanding information content and scope. We here focus on relating two ontologies: the Gene Ontology (GO), which encodes canonical gene function, and the Mammalian Phenotype Ontology (MP), which describes non-canonical phenotypes, using statistical methods to predict GO functional annotations from existing MP phenotype annotations.
Our approach is to define rules for predicting gene function by examining the emergent structure and relationships between the gene functions and phenotypes. The algorithms inspect relationships among multiple phenotype terms to deduce if there are cases where they all arise from a single gene function. We apply this methodology to data about genes in the laboratory mouse that are formally represented in the Mouse Genome Informatics (MGI) resource. From the data, 7444 rule instances were generated from five generalized rules, resulting in 4818 unique GO functional predictions for 1796 genes. As well as creating inferred annotations, our method has the potential to allow for the elucidation of unforeseen, biologically significant associations between gene function and phenotypes that would be overlooked by a semantics-based approach.

This research was supported by NIH NHGRI grant HG-002273 for the Gene Ontology Consortium. JAA internship funding was provided by NIH NHGRI grant HG-007053. We would like to thank the Jackson Laboratory Summer Student Program for their outstanding support.

P-61: OncoCL-KB, a cancer cell knowledgebase

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OncoCL-KB is a knowledgebase built upon the semantics of our previously developed ontology for describing cancer cell types, OncoCL. OncoCL provides a framework for bringing disparate data together in a structured way, such that a morphologic entity as defined by conventional pathology is 'anchored' within cancer progression, associated with its canonical biological counterpart/origin and linked to the molecular genetic abnormalities that characterize it along with the features and properties imparted by the corresponding disrupted cellular pathways, for example, escape from growth regulators or evasion of apoptosis.

OncoCL makes use of a number of other mature biomedical and clinical ontologies. The cell type ontology, CL, describes normal cell types and was not designed to capture the pathology of cancer cells. OncoCL builds upon CL, as a canonical cell (represented in CL) undergoes oncogenic change and tumorigenesis with the acquisition of the cancer hallmarks described by Hanahan and Weinberg. Cellular phenotypes are described using the Phenotypic Quality Ontology (PATO) and the Gene Ontology (GO) and cellular location (normal or metastatic) is described using the anatomy ontology, UBERON.

OncoCL-KB embeds annotated data sets – including cancer-associated genes and genomic variants, cancer-associated pathways, cancer stem cell markers, and cancer mouse models – in the OncoCL semantic framework that has been checked for logical consistency and valid inferential structure. We hope that OncoCL-KB, through the synthesis of complex, heterogeneous data related to cancer cells and cancer progression, will provide a resource that will contribute to a better understanding of cancer predisposition, diagnosis, and treatment.

This work is supported by NIH with funding through NCI CA155825.

P-62: Deep genome sequencing and variation analysis of 10 inbred mouse strains

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The Mouse Genomes Project is an ongoing collaborative effort to sequence the genomes of the common laboratory mouse strains, cataloging all forms of molecular variation, and to produce full chromosome sequences of each strain. In 2011, the initial analysis of sequence variation across 17 strains revealed approximately 56.7M unique SNPs and 8.8M indels. In 2014, we completed deep sequencing (40-80x coverage) of 10 additional inbred strains (BUB/BnJ, C57BL/10J, C57BR/cdJ, C58/J, DBA/1J, I/LnJ, MOLF/EiJ, NZB/BlNJ, NZW/LacJ, and SEA/GnJ) cataloging molecular variation within and across each strain. These strains include important models for immune response, autoimmune abnormalities, leukemia, age-related hearing loss, and rheumatoid arthritis. Approximately, 24.9M unique SNPs were identified across these strains compared to the C57BL/6J reference genome (GRCm38). Similarly, 5M unique indels were identified across all of the strains. When compared to the initial catalog from the initial 17 mouse genomes, these 10 additional strains add approximately 5.6M novel SNPs and 2M short indels. As expected, the C57BL/10J strain showed the least amount of variation compared to the reference genome. This may be due to the shared common origin between the two strains. Interestingly, the most variation within a single strain compared to the reference genome was identified in the Japanese wild-derived MOLF/EiJ strain (12.5M unique SNPs and 2.1M short indels). MOLF/EiJ mice are genetically distinct from common laboratory mice for a number of complex phenotypes and represent a valuable resource for genetic mapping and evolution studies.

P-63: The INFRAFRONTIER Research Infrastructure and the European Mouse Mutant Archive (EMMA)

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INFRAFRONTIER is the European Research Infrastructure for phenotyping and archiving of model mammalian genomes. The INFRAFRONTIER Research Infrastructure provides access to first-class tools and data for biomedical research, and thereby contributes to improving the understanding of gene function in human health and disease using the mouse. The INFRAFRONTIER network currently consists of 23 partners, is engaged in several EC funded projects such as INFRAFRONTIER-I3, InfraCoMP and BioMedBridges, and contributes to the International Mouse Phenotyping Consortium (IMPC).

The core services of INFRAFRONTIER comprise systemic phenotyping of mouse mutants in the participating mouse clinics, archiving and distribution of mouse mutant lines by the European Mouse Mutant Archive (EMMA) and providing access to the NKI's unique archive of embryonic stem cells derived from validated genetically engineered mouse models (GEMM-ESCs) of cancer. In addition, INFRAFRONTIER offers specialized services such as the generation of germ-free mice (axenic service) and training in state-of-the-art cryopreservation and phenotyping technologies. Reduction and refinement to improve animal welfare are among the major goals of INFRAFRONTIER's technology development programme.

The EMMA branch of INFRAFRONTIER 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. At present EMMA holds 3400 mouse strains, corresponding to transgenic mice, induced mutants, gene-traps, knock-ins, knock-outs and also including targeted alleles from Deltagen and Lexicon, as well as strains produced from the International Mouse Knockout Consorium (IKMC) resource. The EMMA network is comprised of 16 partners from 13 countries who operate as the primary mouse repository in Europe. EMMA is funded by the partner institutions, national research programmes and the European Commission's FP7 Capacities Specific Programme. Information on mouse strain submission and ordering and all other services offered by INFRAFRONTIER can be accessed online at www.infrafrontier.eu.

P-64: *Thy1* (CD90) regulates adipogenesis and is decreased by the environmental obesogen Tetrabromobisphenol-A

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Obesity has risen drastically over the last 30 years worldwide. Obesity occurs through excessive adipogenesis (adipocyte formation) or an increase in adipocyte size. Adipocytes produce cytokines such as IL-6 that promote inflammatory diseases. Environmental toxicants termed obesogens such as Tetrabromobisphenol-A (TBBPA) disrupt the endocrine system and increase adipogenesis; however the mechanism(s) remain unknown. THY1 (CD90), a cell surface protein, is a member of the immunoglobulin family. We have previously shown that fibroblasts are heterogeneous for *Thy1* and only THY1$^-$/low fibroblasts can form adipocytes. We hypothesized that TBBPA promotes adipogenesis by decreasing *Thy1* expression due to an increase of endogenous microRNAs. We used mouse 3T3-L1 pre-adipocytes as a model to determine if TBBPA decreases the expression of *Thy1* to promote fat cell formation. We measured *Thy1* mRNA levels by qPCR and THY1 protein levels by western blot. Adipogenesis was measured by inspection of lipid droplets and expression of the key adipocyte marker, FABP4. Our data show that TBBPA decreased the expression of *Thy1* in 3T3-L1 cells and is reproducible in human mesenchymal stem cells. Furthermore, TBBPA treated cells also expressed higher levels of certain microRNAs and FABP4, supporting the concept that loss of *Thy1* alters cell fate. TBBPA exposure for one week before adipogenic induction resulted in a further reduction of *Thy1* and additional increases in FABP4. Thus, prolonged or developmental exposure to TBBPA may affect *Thy1* levels and alter physiology long after exposure, which may contribute to the obesity epidemic.

P-65: Interactive matrix views to access and explore expression information in the Mouse Gene Expression Database (GXD)

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The mouse Gene Expression Database (GXD) integrates data from RNA in situ hybridization, immunohistochemistry, knock-in reporter, RT-PCR, northern, and western blot experiments, with particular emphasis on mouse development, and makes these data freely accessible to a wide variety of database searches (see abstract by Finger, et al.). Expression patterns (i.e. the time and space of gene expression) are annotated in a standardized way by using a detailed anatomical ontology that represents the mouse anatomy hierarchically for each developmental stage, thus allowing the integrated description of expression patterns from experiments with differing spatial resolution. Currently, the mouse developmental ontology comprises more than 20,000 time-specific anatomical structures. To facilitate the exploration of search results in GXD, we have developed two new types of interactive data summaries: tissue-by-developmental stage and tissue-by-gene matrix views. Tissue-by-developmental stage matrices provide high-level overviews of the spatio-temporal expression patterns of genes. Tissue-by-gene matrices enable a comparison of expression patterns. Both types of matrices can be expanded and collapsed along the tissue axis, based on the hierarchical organization of the anatomy. Interactive data filters can be applied to iteratively refine the data set. These new matrix views provide researchers with intuitive high-level summaries of expression results from which they can drill down to more detail, thus facilitating the handling and understanding of extensive and biologically complex expression information. An integral component of the larger Mouse Genome Informatics (MGI) resource, GXD is freely available at www.informatics.jax.org/expression.shtml. GXD is supported by NIH grant HD062499.

P-66: Visualizing the mouse genome

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A primary mission of the Mouse Genome Informatics (MGI; www.informatics.jax.org) database project is to integrate biological annotations associated with mouse genes and proteins with large-scale sequence data sets and with the reference genome for the laboratory mouse. One of the best mechanisms for exploring these data is through interactive graphical displays. We have implemented a powerful new browser called JBrowse. JBrowse is the latest genome visualization tool developed by the Generic Model Organism Database (GMOD) project. JBrowse has been implemented using JavaScript resulting in significantly improved speed for rendering annotation tracks in a web browser window. Compared to MGI's previous genome browser, JBrowse has more flexible options for user interactions and data visualization. The architecture of JBrowse allows the MGI team to update data more frequently and to make available to the community larger data files that are typical of most genome-centric experiments. Even very dense data sets such as SNPs can be rendered without significant delay. Users can easily download the annotations in a particular genome region. Callback functionality in the software has been expanded to give developers the ability to add custom context menus and visualizations on a per track basis. The MGI installation of JBrowse includes data acquired from external annotation providers as well as MGI-specific annotations for phenotype and function. Since multiple genomes can be contained within a single JBrowse instance MGI's JBrowse includes the human genome reference assembly and NCBI's human genome annotations. We anticipate that JBrowse will serve the mouse research community's need to work with the genome assemblies for multiple strains of mice and will present results of test data from complete mitochondrial genomes and "pseudo-assemblies" of mouse strains generated by alignment of large-scale sequence data from multiple strains to the C57BL/6J reference genome. This work is supported by NIH NHGRI HG000330.

P-67: Towards a high-throughput morphological analysis of embryonic lethal knockout mice

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The International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org) whose goal in five years is to phenotype 5000 knockout mouse lines represents an enormous resource to better understand the relationship between gene and phenotype. However, it is estimated that 30% of the lines will be adult lethal (no progeny at P7), creating an opportunity to collect embryonic morphological data on those lethal lines. Recommendations from the Bloomsbury Report (Dis. Model. Mech. 2013 6:571-579) have been adopted by many of the phenotyping centres and 3D imaging will be captured at E9.5, E14.5, E15.5, or E18.5 based on line viability, however many lines will have more extensive data generated from a triage pipeline. At MRC Harwell, around 150 of the IMPC lines will go through an embryonic lethal triage pipeline. Using these lines as real phenotypic test data our goal is to then rapidly and accurately identify 3D morphological phenotypes of approximately 1000 mice lines. This will be achieved using new developments in atlas-generation and morphometric shape analysis to distinguish both subtle and gross morphological changes between wild type and mutant. We will show a number of initial results from the computational processes on unseen novel phenotypes. This will result in the identification of novel genes to better understand the genetics of mouse embryogenesis and also provide benefits to human health with new mouse models of human congenital diseases. This project is part of the MPI2 consortium and is supported by the NIH Common Fund Mechanism: U54 HG006370.

P-68: Recent Enhancements at MGI: Cluster membership, Gene Interaction, and Mutation to Gene Relationships

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Mouse Genome Informatics (MGI) is the international database resource for the laboratory mouse, providing integrated genetic, genomic, and biological data to facilitate the study of human health and disease.

Recent additions to MGI include a more robust representation of relationships between genes/genome features. Three types of relationships are currently supported: gene cluster membership, gene and genome feature interactions, and relationships between alleles/mutations that involve multiple genes.

Cluster membership is visible from gene detail pages. Individual cluster member pages (e.g., *Hoxa1*, *Hoxa2*, etc.) link to the cluster detail page (e.g., *Hoxa* cluster) and vice versa. The cluster page also provides the ability to forward all cluster member genes to the MGI Batch Query to retrieve information about all members of the cluster. Clusters are currently restricted to co-localized and related genes.

Interactions can be viewed from the new "Interaction" section on gene detail pages. The first data set supported describes predicted and verified gene targets of known mouse microRNAs, as these are genes whose expression is potentially regulated by the microRNAs. The interaction section links to a new "Interaction Explorer" page where you can view and filter all of the interactions involving the gene.

Allele detail pages for alleles and genomic mutations that involve multiple genes now display links to all the involved genes. Gene detail pages also now include reciprocal links back to these alleles.

MGI is freely available at www.informatics.jax.org. The work described here was supported by NHGRI grant HG000330.

P-69: Representing Homologs in the Mouse Genome Informatics System

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A core element of the Mouse Genome Informatics (MGI, www.informatics.jax.org) resource is the representation of comparative genetic and genomics data. In particular, MGI presents homology data among the vertebrates and uses this information to provide integrated representation of disease and phenotype data between mouse and human.

MGI has implemented a revised representation of homology that results in the inclusion of in-paralogs in the homology gene sets. Currently, these data are brought into MGI from Homologene (www.ncbi.nlm.nih.gov/homologene). However, there are several informatics resources that use different algorithms for predicting ortholog sets. HCOP (www.genenames.org/cgi-bin/hcop), a resource developed by the HUGO Human Gene Nomenclature Committee, searches EggNOG, Ensembl, HGNC, HomoloGene, Inparanoid, OMA, OrthoDB, OrthoMCL, Panther, PhylomeDB, TreeFam and ZFIN for homology assertions and displays these for each human gene entry. At NCBI, gene detail pages link to several resources including Homologene, a set of orthologs from NCBI's Annotation Pipeline, and to the OrthoDB resource.

Here we discuss some of the complexities and challenges of representing homology data in MGI, and the impact of these complexities on developing search algorithms and integrated displays of comparative data between mouse and human.

This work is supported by NIH NHGRI grant HG000330.

P-70: Detecting Genome Variants using msBWT

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Next Generation Sequencing technology has tremendously increased the throughput of sequence data. Traditionally, a sequence aligner will be used to map reads from an NGS dataset onto the reference genome, and then a post-process is used to detect variants between the alignments and the reference genome. However, alignment can be very time consuming and inaccurate if the sample has many variants with respect to the reference genome. To avoid this bottleneck, we introduce an alignment-free approach to calling variants based on using the query capabilities of the Multi-String Burrows Wheeler Transform (msBWT).

Using msBWT, the times of occurrence for a k-mer can be gained from the dataset in O(k) time which is independent from the size of the dataset. We slide a window of size 40bp with overlapping of 20bp from the genome to generate a sequence of k-mer counts, i.e. read depths from a dataset. If two consecutive windows have a much lower depth than the two sides around them, we'll assume there is a variant in the 20-mer of overlapping region between the two windows. Then we'll recall reads covering both of the two sides 20-mer around the low-depth 20-mer. A consensus sequence will be generated from those reads, resulting in the calling of variants after aligning the consensus to the reference. We present a targeted web search tool to correct the reference genome using this method at http://csbio.unc.edu/CEGSseq/index.py?run=msPileup.

P-71: Building the First Functional Catalogue for a Mammalian Genome: Informatics for the International Mouse Phenotyping Consortium

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The **International Mouse Phenotyping Consortium** (IMPC) is building the most comprehensive functional catalogue of a mammalian genome, which will give new insights into disease. The IMPC is coordinating efforts to generate a knockout mouse strain for every protein-coding gene. These mouse strains are characterized using a standardized, broad-based phenotyping pipeline and data is collected and archived centrally by the IMPC-Data Coordinating Centre. Dedicated 'data wranglers' are working with each phenotyping centre to ensure proper transfer and quality control of data. An automated statistical analysis pipeline identifies knockout strains with significant changes in phenotype parameters. Potential disease models are identified by orthologous gene and by orthologous phenotype features. Over 4000 IMPC mouse strains have been produced, with phenotype data available for over a thousand of these strains. Users can freely access all data including new gene-phenotype via an intuitive web portal. Annotation with biomedical ontologies allows biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research. Users can register interest in genes so they may be informed as mouse models and new phenotype data become available. The community is invited to explore and provide feedback as we build this rich resource for into disease mechanisms at:

www.mousephenotype.org

P-72: The FaceBase Cre Driver Project: new tools for orofacial clefting research

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Cleft lip and cleft palate are among the most common birth defects in the human population, with a prevalence of approximately 1 in 700 live births. Surgical repair of clefting is well established, however requires multiple procedures and significant economic cost. Genetic factors are suspected in 25-50% of cases, however the genes and interactions required for palate shelf growth, elevation, and fusion are poorly defined and understood.

The advent of conditional gene targeting via the cre/loxP system has enabled precise manipulation of the genome *in vivo*. Large repositories of floxed alleles, most prominently those developed under the International Knockout Mouse Consortium (IKMC), are rapidly becoming available and will target a large proportion of mouse genes. Tissue restricted and temporally restricted cre driver strains are needed to fully apply these new resources in hypothesis driven experiments.

To this end, as part of the FaceBase Consortium, we have generated a set of cre driver strains to expand the "tool kit" available to the craniofacial research community. We have worked with members of the community to select drivers that fill gaps in the existing resources, with a specific emphasis on tools suitable for studying the mid-face and palate. Approaches include both BAC transgenics and targeted knock-in alleles, and both constitutive and inducible versions of cre were employed. Each resulting line is characterized for cre activity in the developing face and head from embryonic day 11.5 - 15.5 using the B6.129S4-*Gt(ROSA)26Sor*^{tm1Sor}/J reporter strain. Lines with expression restricted to relevant structures are made available through the JAX repository. All characterization data is published on both the FaceBase Consortium website and the JAX CrePortal website.

P-73: An Update on the Distribution Center for the Collaborative Cross Population at UNC-Chapel Hill

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The Collaborative Cross (CC) is a panel of recombinant inbred lines derived from eight genetically diverse laboratory inbred strains. The genome of each available line is determined by computing the union of the MRCAs (Most Recent Common Ancestors) of the line and the genomes of each line are accessible at http://csbio.unc.edu/CCstatus/index.py. These genomes identify the founder haplotype in the regions of the genome that are fixed and the alternating haplotypes in the regions that are potentially segregating. New publications are coming out using specific CC lines and their use as disease models, i.e. colitis, Ebola, influenza. Here we provide a brief description of the status of the CC in the US and distribution efforts by the UNC at Chapel Hill Systems Genetics Core Facility. Specifically we will discuss: 1) the number of lines available; 2) the improvement in the genome reconstruction and the 36 states of probabilities using the 75,000 SNP array called MegaMUGA (see Kao, *et al.*); 3) Rederivation and archiving; and 4) Distribution and use.

P-74: Analysis of sleep and wake in the JAX KOMP2 phenotyping pipeline using a non-invasive, high-throughput piezoelectric system

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The present study employs a noninvasive, high-throughput system to characterize sleep- and wake-related traits in a large population of control and single-gene knock-out mice. A piezoelectric sensor placed at the bottom of the mouse cage records gross body movements, while an automatic classifier analyzes signal features to identify sleep and wake. The system characterizes sleep time over 24 hours, as well as during the light and dark phase, the distribution of sleep bout length across those intervals, crepuscular activity, the time of peak wake, and several other traits. The classifier correlates approximately 95% with EEG and 90-95% with human observation. This system has been used in a variety of studies, including quantitative trait loci mapping of sleep-related traits, as well as characterizing sleep in mouse models of Alzheimer's disease, traumatic brain injury, and other disorders. We are developing the system to differentiate REM from NREM sleep.

We created a data confidence metric for analysis of sleep data generated in this system to provide a well-defined signal quality measure for the JAX Knockout Mouse Phenotyping Program (JAX-KOMP2), which aims to characterize a variety of morphological, physiological and behavioral phenotypes in single-gene knock-out mouse lines. The piezoelectric sleep phenotyping system is a unique part of The Jackson Laboratory's KOMP phenotyping pipeline. We present the results of the sleep phenotyping performed so far, including significant differences by sex and genotype in over 600 C57BL/6NJ controls and dozens of single-gene knockout lines. This study provides a large data set of sleep phenotype information, as well as identifying candidate genes that influence sleep. Data will also be compared to other phenotyping paradigms in the pipeline to inform about additional measurable traits in the pipeline coincident with sleep.

P-75: Quantifying allele-specific alternative splicing in personalized genomes using EM algorithm

(See abstract SO-12 in the student/postdoc session)

P-76: Mining MGI Data Using MouseMine

Joel Richardson[*], Howie Motenko, Steven Neuhauser, and Michael O"Keefe

The Jackson Laboratory

The Mouse Genome Informatics (MGI) project at The Jackson Laboratory collects, integrates, and disseminates information about the laboratory mouse (http://www.informatics.jax.org). MouseMine is a powerful new tool for accessing MGI data (http://www.mousemine.org). Built on the InterMine data warehouse framework (http://www.intermine.org), MouseMine provides: (1) access to the core data and annotations from MGI (gene and allele catalog, gene expression data, strains and models, function, phenotype, and disease annotations, cross references); (2) numerous “canned” queries; (3) the ability to modify and refine any query dynamically or to compose a new query from scratch, using a point-and-click interface; (4) the ability to download any query result in a variety of formats or to forward the results to Galaxy or GenomeSpace; (5) the ability to create and save lists of objects, by uploading ids or by saving objects returned by queries; (6) the ability to combine lists (intersection, union, difference) and then use those lists to drive further queries to hone their results; (7) support for both anonymous and authenticated usage (logged in users can save lists and customized queries permanently); and finally, (8) the ability to access all the aforementioned functionality via RESTful web services. This poster illustrates one example of how MouseMine enables powerful querying via a sequence of simple steps.

MouseMine is supported by NIH grant HG004834.

P-77: Looking for Mice in All the Wrong Places? Try The International Mouse Strain Resource (IMSR)-- the Place to Go to Find the Right Mouse

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The International Mouse Strain Resource (IMSR) exists to help the international biomedical research community find and obtain mouse resources for research. IMSR is a free, searchable online database of mouse strains, stocks and mutant ES cell lines, including many generated by large-scale targeted and ENU mutagenesis projects, available from repositories worldwide. Currently >28,200 strains and >210,000 ES cell lines are included in the IMSR catalog.

You can search the IMSR using various parameters: any part of a strain name, gene/allele symbol or name; the maintained State of the stock (live, cryopreserved embryos or gametes, or ES cell lines); the Strain Type (*e.g.*, coisogenic, inbred, recombinant inbred); the Repository or geographic region; and the Mutation Type (*e.g.*, chemically induced, spontaneous, targeted). You also can access IMSR records from a Mouse Genome Database (MGD) Allele Detail page.

Information in the IMSR about a strain includes the Strain Name and any Synonyms (*e.g.*, from the literature); the Strain Type; any mutations or transgenes it carries; the Mutation Type(s); the Repository where it is maintained; and the State(s) in which it exists. Each strain record may contain links to the MGI Allele and Gene Detail pages and to the strain description and order form on the repository's Web site; a click opens an email form addressed to the repository.

Search results can be filtered by State, Strain Type, Provider (Repository) and Mutation Type. The results can be sorted by Strain Name or Repository. They also can be exported in either tab-delimited or Excel format.

Repositories, commercial vendors, and institutional mouse facilities that serve the general research community may be included in the IMSR. The first step is to register your facility with IMSR, whereupon you will be contacted by an IMSR staff member to discuss the strain submission process.

Supported by NIH grant HG000330.

P-78: High-density lipoproteins mediate microRNA intercellular communication in type 2 diabetes.

(See abstract SO-13 in the student/postdoc session)

P-79: High-Throughput Mouse Phenotyping will uncover new insights into Immune Mechanisms

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The International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org) is an international effort to phenotype up to 20,000 mutant mouse lines. By generating a knockout strain for each protein-coding gene, the phenotyping relies on assessing each mouse according to a pipeline of standard operating procedures - including Immunophenotyping.

Stemming from the EUROPHENOME project, the Immunophenotyping protocol has been established with an unprecedented agreement between eight core phenotyping centres, expanding to 11 in the future. The agreement on this protocol covers two panels of different populations and sub-populations of cells, markers, reagents and all steps necessary to perform the flow cytometry runs that will generate all the analysis data. The IMPC's Data Coordination Centre (DCC) has teamed with FlowRepository to provide storage for flow cytometry data files, and the extra benefit of performing automatic analysis, in supervised or unsupervised fashion. Data flow and analysis will be streamlined, to allow fast access to data and statistical results, leading to publication. All information will be available for free on the IMPC portal.

Data generated from the Immunophenotyping protocol in particular, and the whole pipeline in general, will provide confirmation of previously known links between mouse phenotypic and genomic data with Human data and genetic diseases, but also reveal new associations. Some centres are developing specialized secondary phenotyping pipelines to more closely study mechanisms related to human infectious and autoimmune diseases, using subsets of IMPC mouse strains. Such an holistic approach potentiates the discovery of several co-founding aspects resulting from a single genetic change.

P-80: Keeping up with the mouse reference genome assembly

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The mouse belongs to a select group of organisms whose genomes are represented by clone-based, finished reference assemblies. The availability of a high quality reference assembly not only promotes studies of mouse biology but allows comparative genome analyses that enable the mouse to serve as an outstanding model organism for the analysis of human development and disease. The mouse assembly is maintained by the Genome Reference Consortium (GRC; http://genomereference.org), the group also responsible for the human and zebrafish reference assemblies. With the release of GRCm38 (mm10) in 2011, the mouse reference assembly adopted a GRC assembly model that allows it to include representation for sequences absent from the C57BL/6J-derived reference chromosomes due to inter-strain variation. These additional sequence representations, known as alternate loci, are strain-specific stand-alone scaffolds given chromosome context by virtue of their alignment to the reference chromosomes. In GRCm38, there 99 alternate loci associated with 70 distinct genomic regions. These alternate loci, which have been annotated by RefSeq, represent 13 different strains and include over 3 Mb sequence not found on the chromosomes. Despite its high quality, the mouse reference assembly still contains sequence errors, misassembled regions and gaps, all of which the GRC is actively working to correct. The GRC assembly model provides a mechanism for the timely distribution of assembly updates between major assembly releases without disruption of chromosome coordinates. Like the alternate loci, patches are stand-alone scaffolds placed into chromosome context by alignment. Fix patches correct existing assembly sequences, while novel patches add sequences from other strains. To date, there have been 3 patch releases for GRCm38, which provide another 1 Mb of unique sequence. We will discuss our ongoing curation efforts, plans for adding sequences from other strains and future assembly releases, as well as GRC resources for staying abreast of assembly updates.

P-81: Czech Centre for Phenogenomics: new research infrastructure for production of mouse models and their phenotyping

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The Czech Centre for Phenogenomics (CCP), a newly set up infrastructure, is combining genetic engineering capabilities, advanced phenotyping and imaging modalities, specific pathogen free (SPF) animal housing and husbandry, as well as cryopreservation and archiving, all in one central location – located within the new BIOCEV complex (Biotechnology and Biomedicine Center of the Academy of Sciences and Charles University in Vestec) and the hosting institution, the Institute of Molecular Genetics (IMG) in Prague.

The Czech Centre for Phenogenomics (CCP), through its memberships in INFRAFRONTIER and the International Mouse Phenotyping Consortium (IMPC), is a partner in a collective global network that aims to comprehensively and systematically analyze the effect of loss of function gene mutations in mice.

Current services in model production include custom targeted mutagenesis and transgenesis, blastocyst injection, pronuclear microinjection, sperm and embryo cryopreservation, *in vitro* fertilization and breeding on demand. Over 80 trangenic generation projects were completed in 2013, from both local and international customers and this number should further increase over next years as we build towards full functionality. Full operation of standardized phenotyping services is expected at the end of 2015, corresponding to the full functionality of the newly constructed CCP building with a floor-space of 7,200 m^2 and maximum capacity of 12,000 cages for mice (30,000 when combined with existing capacity) and 4,000 cages for rats, and will have over 70 employees.

P-82: Evaluating the ENU landscape and its impact on ageing phenotypes.

(See abstract SO-14 in the student/postdoc session)

P-83: Mutant Mouse Resource and Research Centers at The Jackson Laboratory

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The Mutant Mouse Resource and Research Centers' (MMRRC) primary responsibility is to facilitate a wide spectrum of research by serving as a reliable, readily-accessible, quality-assured source of mutant mouse strains and ES cells. As a national consortium of breeding, cryopreservation and distribution facilities, MMRRC members include: The Jackson Laboratory, University of Missouri, University of North Carolina at Chapel Hill and University of California at Davis.

The JAX-MMRRC houses several major collections of strains including the Keck miRNA lines, a set of 42 small (micro) RNA conditional knockout mice and ES cell lines from the McManus Laboratory at the Gladstone Institute. The Pleiades Promoter Project and CanEuCre collections include a set of 85+ mouse lines utilizing human DNA MiniPromoters that drive region- and cell specific gene expression in the mouse brain. Pleiades MiniPromoters drive beta-galactosidase (lacZ) and EGFP reporter expression, while CanEuCre lines use human MiniPromoters to drive a tamoxifen-inducible, Cre recombinase (icre/ERT2).

The JAX-MMRRC houses an extensive collection of mouse strains related to Alzheimer disease. APPswe/PS1dE9 (Tg(APPswe,PSEN1dE9)85Dbo) is a transgene expressing a chimeric mouse/human amyloid precursor protein and a mutant human presenilin 1 directed to CNS neurons. Both mutations are associated with early-onset Alzheimer's disease. 3xTg-AD mice exhibit plaque and tangle pathology associated with synaptic dysfunction. 5XFAD transgenic mice overexpress mutant human *APP*(695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations along with human *PSEN1* harboring two FAD mutations, M146L and L286V.

The research component of this project has developed protocols allowing the efficient derivation of ES cells from various strain backgrounds. Additionally, novel ES cell lines were derived from a number of MMRRC strains (especially from Alzheimer's disease models) primarily for *in vitro* phenotypic analysis.

The JAX-MMRRC is supported by the Office of Research Infrastructure Programs/OD (grant number OD010921) of the NIH.

P-84: *Zic3* is a novel, *in vivo* inhibitor of β-catenin/TCF mediated transcription

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During murine gastrulation, the canonical Wnt/β-catenin pathway plays a vital role in formation of primitive streak, the progression of gastrulation and tissue patterning along the anterior-posterior axis. A genetic screen for mutations that affect embryogenesis identified a mouse strain, katun (*Ka*), in which mutant embryos exhibit incompletely penetrant, partial (posterior) axis duplications and anterior truncation. Both of these phenotypes are redolent of elevated canonical Wnt signalling and analysis of *Ka* embryos reveals ectopic expression of direct targets of Wnt/β-catenin mediated transcription in the forebrain of mutant embryos. The katun mice carry a nonsense mutation in the zinc finger of the cerebellum 3 (*Zic3*) gene that generates a null allele. This gene is a member of the Zic family of transcriptional regulators and previous work has shown that ZIC proteins can inhibit β-catenin/TCF mediated transcription when overexpressed in cell lines. ZIC proteins physically interact with TCF proteins, but not β-catenin, and do not contact DNA to inhibit β-catenin/TCF mediated transcription. The katun mutant protein cannot inhibit β-catenin/TCF mediated transcription. To determine whether *Zic3* inhibits canonical Wnt signalling *in vivo*, $Zic3^{Ka}$ mice were crossed with the batface (*Bfc*) activating allele of β-catenin ($Ctnnb1^{Bfc}$). This increases the penetrance and severity of Wnt-associated phenotypes, providing the first evidence that the ZIC proteins function as novel *in vivo* Wnt inhibitors. In mouse and human, mutation of *Zic3/ZIC3* is associated with heterotaxy, a disorder of left-right axis formation. Analysis of the *Ka* and *Bfc* strains indicates that dysregulated Wnt signalling may contribute to the development of heterotaxy in humans.

P-85: Transcriptional deconvolution of meiotic substages using RNA-seq and cytological analysis of the first wave of spermatogenesis in the mouse

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Spermatogenesis is a complex series of events that are initiated continuously in mammals. For these reasons, substages of meiosis have frequently been studied during the first wave of spermatogenesis, which is semi-synchronous. Most analyses of the first wave have relied on decades-old cell composition analyses based on histological data, which were limited by a paucity of markers for meiotic substages. This study was undertaken to provide a detailed comparison of the relationship between the meiotic transcriptome and cell populations more precisely defined by molecular cytological analysis of protein markers for the substages of meiosis. A robust experimental design allowed computational decomposition of transcription patterns based on meiotic substages. Germ cells were enriched from the testes of C57BL/6J mice at 8, 10, 12, 14, 16 and 18 days post-partum, with 5 biological replicates at each age. Germ cells obtained from each mouse were used for surface-spread chromatin preparations, which were immunolabeled for stage-specific marker proteins (including STRA8, SYCP3, phosphorylated histone H2AFX, and histone HIST1H1T). This allowed discrimination of spermatogonia, pre-leptotene, early leptotene, late leptotene, zygotene, early pachytene, late pachytene, and diplotene spermatocytes (8 cell populations). RNA-sequencing (RNA-seq) libraries were constructed from the same germ cell samples, in order to examine temporal patterns of gene expression. Parallel principal component analyses of both gene expression and fine-grain cytological data similarly separated samples, providing strong evidence for meiotic prophase substage-specific gene expression patterns. For each gene, a stepwise forward regression model determined which substage(s) best explained the gene's expression. Based on these models, we constructed substage-specific gene lists, and parsed these into substage-specific functions with gene ontology annotations. This study has not only provided a finer-grained analysis of the shifting cell populations during the juvenile onset of spermatogenesis, but also has validated genome-wide computational approaches to resolve the complexity of the meiotic transcriptome.

P-86: Embryonic stem cells from mouse models of aneuploidy syndromes.

Anne Czechanski*, Candice Byers, Cathleen Lutz, and Laura Reinholdt

The Jackson Laboratory

The introduction of site specific nuclease gene editing has enabled one step generation of genetically engineered mice, however, mouse embryonic stem cells (mESCs) continue to be key reagents for complex genome engineering, *in vitro* differentiation and development of stem cell based therapies, and are essential for basic research on pluripotency and early lineage commitment. We developed an optimized mESC derivation and characterization protocol that allows for the generation of mESC from any strain, including those previously classified as recalcitrant. To date, we have created novel mESC lines from over 50 strains and over 40 of our mESC lines are now available to the scientific community through the The Jackson Laboratory (JAX) and through the Mouse Mutant Resource and Research Center at JAX. Now, with support from the Mouse Models of Cytogenetic Disorders repository at JAX, we have completed the derivation of mESC lines from mouse models of Turner and Down syndromes. We present here our initial characterization and genomic analysis of these mESC lines. Because the lines were derived from reciprocal crosses, they will not only provide *in vitro* tools for disease modeling; they will also provide a platform for establishing the role of parent of origin in aneuploidy syndromes.

P-87: A Mouse Embryonic Phenotyping Platform for Understanding Developmental Disorders

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Detailed characterization of embryonic phenotypes in the mouse is essential to understanding the genetic foundations of human congenital birth defects, developmental abnormalities and the developmental origins of adult disease. To extend the development of mouse models of human disease to these important classes we have developed a high-throughput, standardized, embryonic phenotyping program. The phenotyping strategy involves a tiered screen to examine embryos at discreet, highly informative, developmental time points; and allows for the determination of the approximate time of lethality and observations of associated embryonic phenotypes. A combination of gross morphological observations and advanced 3-dimensional imaging modalities, including iodine-contrast microCT and optical projection tomography (OPT), are employed to assess embryological phenotypes. All phenotypes are scored using standard Mammalian Phenotype (MP) terms to facilitate database interrogation.

This platform is being employed to characterize strains produced and phenotyped as part of the Knockout Mouse Phenotyping Program (KOMP2), which aims to generate and phenotype a genome-wide collection of knockout mice. Roughly 30% of these strains are predicted to be embryonic lethal, and the embryonic phenotypes of these strains are a rich source of functional information. As part of this program, we have already identified over 60 lethal and sub-lethal genes, which are now in the Embryonic Phenotyping program. In addition, we have extended our analysis to mouse models of human developmental disorders, generated at high-throughput using CRISPR/Cas9 genome-editing technology. An overview of our pipeline and current progress, including examples of morphological abnormalities observed in embryonic mutants will be presented.

P-88: Investigating and deriving induced pluripotent stem cells from the non-permissive NOD/ShLtJ and WSB/EiJ strains

(See abstract SO-04 in the student/postdoc session)

P-89: Evaluation of carry-over effects in the high-throughput JAX KOMP2 adult phenotyping pipeline

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The Knockout Mouse Project (KOMP) and International Knockout Mouse Consortium (IMPC) have generated ES cell lines with single gene knockouts covering the mouse genome. These cell lines are distributed for rederivation and high-throughput phenotype screening.

One challenge in replicating the results of high-throughput phenotyping platforms in further characterization of mutant mice is the potential influence of repeated testing. In this so called 'carryover effect' the test outcome is affected by prior test experience which could bias effect size estimation or otherwise influence the reproducibility of results.

The Jackson Laboratory has a comprehensive adult phenotyping pipeline with assays covering multiple physiological, behavioral and morphological characteristics. We performed a C57BL/6NJ carry-over study to determine whether repeated testing influences phenotypic observations. We matched C57BL/6NJ KOMP controls, which receive every test in the pipeline, by age and sex to naïve C57BL/6NJ mice for each test. We fit a fully balanced simple linear regression model to test whether the effect of naïve mice is the same for mice that went through the pipeline and further computed a least squares mean comparison to determine sex effects.

We observed significant carryover effects in a very small number of the phenotypic measures for both behavioral and physiological tests; few of which reveal an effect due to the interaction of sex and carryover.

Although these effects are infrequent, researchers will need to consider the effects of prior handling and testing in the further extrapolation of certain test-battery derived phenotypes.

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