The 27th International Mammalian Genome Conference

Colegio Fonseca, Salamanca, Spain
September 15-18, 2013

Full Conference Program

Saturday, 14th September 2013

16.00 – 20.00	Secretariat Meeting	Lazarillo Room at the Abba Fonseca

Sunday, 15th September 2013

09.00 – 20.00	Registration Open	Hospederia Fonseca
10.00 – 13.00	Bioinformatics Workshop (to include mid-morning tea/coffee, but lunch is NOT included)	Sala Menor
14.30 – 19.00	**Student Conference** Session Chairs: David Beier and Teresa Gunn (Lunch NOT included)	Sala Menor

14.30 SO-1: SINEUPs: Novel Antisense ncRNA Can Enhance Protein Translation
Hazuki Takahashi, Division of Genomics Technologies, RIKEN, Yokohama, Japan

14.45 SO-2: X Chromosome Inactivation Choice: Beyond the X Chromosome-Controlling Element
John Calaway, UNC at Chapel Hill, Chapel Hill, NC

15.00 SO-3: Comparison of Meiotic Recombination Rates Using Immunostaining and Chromosomal Painting
Courtney Vaughn, North Carolina State University, Raleigh, NC

15.15 SO-4: Starting at the Ends: High-Resolution Sex-Specific Linkage Maps of the Mouse Indicate That Recombination in Male Germline Progresses from Telomeres to Centromeres
Andrew Morgan, UNC at Chapel Hill, Chapel Hill, NC

15.30 SO-5: Defining the Role of Mouse COPII Component SEC24C
Elizabeth Adams, Human Genetics Dept. University of Michigan, Ann Arbor, MI

15.45 SO-6: A Systems Biology Approach to Identify Interactions between Breast Cancer and Ageing
Maria del Mar Saez Freire, Centro de Investigacion del Cancer IBMCC. Centro Mixto CSIC/Universidad de Salamanca

16.00 SO-7: A Novel Role for an Olfactory Receptor in Pheromone Communication
Andrew Bard, Wellcome Trust Sanger Institute, Cambridge, UK

16.15 – 16.45	Break	Hall Auditorio

16.45 SO-8: Inferring Founder Probabilities in Admixed Animals Using Genotyping Intensities
Chen-Ping Fu, UNC at Chapel Hill, Chapel Hill, NC

17.00 SO-9: Analysis of the Host Response to Influenza A Virus Infection in the Collaborative Cross Founder Strains
Sarah Leist, Helmholtz Centre for Infection Research, Braunschweig, Germany

17.15 SO-10: Identification of Tumor Genetic Determinants of Response to Anthracyclines and Taxanes in an *Erbb2* Breast Cancer Mouse Model
Adrian Blanco Gomez, Centro de Investigacion del Cancer IBMCC. Centro Mixto CSIC/Universidad de Salamanca

17.30 SO-11: *Lman1* Deficient Mice Reveal New Insights Into Coagulation Factor V and VIII Secretion
Lesley Everett, Human Genetics Dept. University of Michigan, Ann Arbor, MI

17.45 SO-12: Systems Pharmacogenomics Approach Identifies Synergistic Molecular Action of Combined MTOR/HDAC Inhibition on MYC
Benjamin Gamache, National Cancer Institute, Bethesda, MD

18.00 SO-13: Determining Role of Innate Immunity in Mice Resistant to Rift Valley Fever
Rashida Lathan, Mouse Functional Genetics, Institut Pasteur, Paris, France

18.15 SO-14: The Metabolic Syndrome in Closely Related Berlin Fat Mouse Inbred lines
Irfan Jumabhoy, King's College London, UK

18.30 SO-15: Exploring Regulatory Networks and Interactions of *Mir96* in the Developing Inner Ear
Morag Lewis, Genetics of Deafness, King's College London, UK

18.45 SO-16: A Sensitized Whole Genome ENU Mutagenesis Screen Identifies a Novel Suppressor Region for Lethal Thrombosis in the Factor V Leiden Mouse
Kart Tomberg, Human Genetics Dept. University of Michigan, Ann Arbor, MI

15.00	Exhibition space open to display posters	Hall Auditorio
16.00 – 18.00	Optional City tour #1	
19.30 – 21.30	Welcome Reception and Tapas (Tuna at 20.00)	Sala de las Pinturas Colegio de Fonseca

Monday, 16th September 2013

08.00 – 18.30	Registration Open	Hospederia Fonseca
09.00 - 09.30	Welcome and Opening Comments	Auditorio
09.30 – 11.00	**Aging and Adult-onset disease modeling I** Session Chairs: Sarah Carpanini and David Threadgill	Auditorio

09.30 O-1: Transposon Mutagenesis Uncovers Evolutionary Forces Driving BrafV600E Melanoma
Neal Copeland, The Methodist Hospital Research Institute, Houston, TX

09.45 O-2: A Metastasis Susceptibility Gene for Estrogen Receptor Negative Breast Cancer Maps to the Distal End of Chromosome 6
Kent Hunter, National Cancer Institute/NIH, Bethesda, MD

10.00 O-3: The Role of *Far2* in the Initiation of Age-Related Kidney Disease.
Ron Korstanje, The Jackson Laboratory, Bar Harbor, ME

10.15 O-4: Dissection Of ERBB2 Breast Cancer Heterogeneity By A Systems Biology Approach
Jesus Perez-Losada, Instituto de Biologia Molecular y Celular del Cancer de Salamanca, Salamanca, Spain

10.30 O-5: Amino Acid Differences Between Mouse and Human Alpha Synuclein Influence Pathogenicity in Yeast and Mice
Deb Cabin, McLaughlin Research Institute, Great Falls, MT

10.45 – 11.15	Break	Hall Auditorio
11.15 – 13.00	**Aging and Adult-onset disease modeling II** Session Chairs: Fernando Pardo-Manuel de Villena and Karen Steel	Auditorio

11.15 O-6: The Harwell Ageing Mutant Screen
Paul Potter, MRC Harwell, Oxfordshire, UK

11.30 O-7: An Aging Screen Identifies Novel Models of Age-Related Hearing Loss
Steve Brown, MRC Harwell, Oxfordshire, UK

11.45 O-8: The Effect of Dietary Deficiencies During Gestation on Adult Behavior and Gene Expression in Mice
Lisa Tarantino, UNC at Chapel Hill, Chapel Hill, NC

12.00 O-9: Multiple QTL Mapping of Cardiac Collagen Deposition in an F2 Population of *Scn5a* Mutant Mice Reveals Interaction Between *Fgf1* and *Pdlim3*, *Gpr158* & *Itga6*
Elisabeth Lodder, Academic Medical Center, University of Amsterdam, The Netherlands

12.15 Come see my poster

13.00 – 16.00	Lunch (Cloisters), Exhibition & Posters (Odd)	Hall Auditorio
13.00 – 14.00	Editorial Board Lunch	Library
16.00 – 17.30	**Trainees and Chapman Lecture** Session Chairs: David Beier and Steve Munger	Auditorio

16.00 S-1: Student Paper
TBA

16.15 S-2: Student Paper
TBA

16.30 **Verne Chapman Lecture**: Harnessing Transposons for Cancer Gene Discovery
Nancy Jenkins, The Methodist Hospital Research Institute, Houston, TX

17.30 – 20.30	Systems Genetics Workshop (optional)	Sala Menor
17.30 - 19.30	Optional City tour #2 (Same sites as #1)	
	Evening free for delegates' own dinner plans (Note most restaurants start serving dinner at or after 9PM)	

Tuesday, 17th September 2013

08.30 – 20.00	Registration Open	Hospederia Fonseca
09.00 – 11.00	**Human disease models I** Session Chairs: Nigel Crawford and Linda Siracusa	Auditorio

9.15 O-10: Studying Mouse Knock-Out Lines for Resistance and Susceptibility to Influenza A Virus Infection
Klaus Schughart, Helmholtz Centre for Infection Research, Braunschweig, Germany

9.30 O-11: Genetic Regulation of *Zfp30*, *Cxcl1*, and Neutrophilic Inflammation in Mouse Lung
Samir Kelada, Department of Genetics, UNC at Chapel Hill, Chapel Hill, NC

9.45 O-12: QTL identification and Confirmation of *Trim55* Contribution to SARS-CoV-Induced Vascular Cuffing Using the Collaborative Cross
Lisa Gralinski, Epidemiology Department, UNC at Chapel Hill, Chapel Hill, NC

10.00 O-13: Impact of Changes in Microbiota Associated with Rederivation of a Mouse Model of Inflammatory Bowel Disease
Craig Franklin, University of Missouri, Columbia, MO

10.15 O-14: Identifying New Antimalarial Targets Using ENU Mutagenesis
Simon Foote, Macquarie University, Sydney, Australia

10.30 Discussion

10.45 – 11.15	Break	Hall Auditorio
11.15 – 13.00	**Human disease models II** Session Chairs: Terry Magnuson and Karlyne Reilly	Auditorio

11.15 O-15: Identification of a Novel ENU-Induced Mutation Affecting Plasma Triglyceride Levels in Mice
Karen Svenson, The Jackson Laboratory, Bar Harbor, ME

11.30 O-16: Positional Cloning of the Chr 15 Quantitative Trait Locus *Fob3b2* Affecting Leanness in Mice
Jasmina Beltram, University of Ljubljana, Slovenia

11.45 O-17: Fine-Mapping a Trans-eQTL Hotspot
Jianan Tian, University of Wisconsin , Madison, WI

12.00 O-18: Combined Sequence-Based and Genetic Mapping Analysis of Complex Traits in Outbred Rats
Amelie Baud, Wellcome Trust Centre for Human Genetics, Oxford, UK

12.15 Come see my poster

13.00 – 14.00	Nomenclature Meeting	Library
13.00 – 16.00	Lunch (Cloisters), Exhibition & Posters (Even)	Hall Auditorio
16.00 – 18.00	**Keynote and Trainee Presentations** Session Chairs: Lluis Montoliu and Karen Svenson	Auditorio

16.00 Keynote Lecture: Secreted Frizzled Related Proteins: From Development to Neurodegeneration
Paola Bovolenta, Spanish Nacional Research Council, Madrid, Spain

17.00 O-19: Caudal Regression in Danforths Short Tail is Caused by the Ectopic Expression of *Ptf1a* Induced by the Insertion of a Transposon
Kei Semba, Institute of Resource Development and Analysis, Kumamoto, Japan

17.15 TBA
Student Presentation

17.30 O-20: A Visualization Tool for Exploring the Gene Expression Landscape in a Full Three-Founder Diallele
J Matt Holt, Dept of Computer Science, UNC at Chapel Hill, Chapel Hill, NC

17.45 O-21: A Large Scale Reverse Genetic Screen to Identify Novel Skin Phenotypes in Mouse Mutants
Kifayathullah Liakath-Ali, King's College London, London, UK

18.00 – 18.30	Break	Hall Auditorio
18.30 – 19.45	**Comparative genomics, Population genetics & Evolution** Session Chairs: Monica Justice and Darren Logan	Auditorio

18.30 O-22: Pervasive Allelic Imbalance Revealed By Allele-Specific Gene Expression In Highly Divergent Mouse Crosses
Jim Crowley, Dept of Genetics, UNC at Chapel Hill, Chpael Hill, NC

18.45 O-23: RNA-seq Alignment to Individualized Diploid Transcriptomes Reveals Extensive Local Genetic Regulation and Differential Allelic Expression in Outbred DO Mice
Steve Munger, The Jackson Laboratory, Bar Harbor, ME

19.00 O-24: The Architecture of Parent of Origin Effects in Mice
Richard Mott, University of Oxford, Oxford, UK

19.15 O-25: Compatibility of *Prdm9* alleles in the (PWD x B6)F1 Sterile Hybrids
Zdenek Trachtulec, Inst.Mol.Genetics ASCR, Prague, Czech Republic

19.30 O-26: A Novel Meiotic Drive System in the Mouse Gives Rise to a Selective Sweep in the Absence of Changes in Fitness
John Didion, Dept of Genetics, UNC at Chapel Hill, Chapel Hill, NC

19.45 – Evening Free for delegates' own dinner plans

Wednesday, 18th September 2013

08.30 – 18.30	Registration Open	Hospederia Fonseca
09.00 - 09.15	Annoucements	Auditorio
09.15 – 10.45	**Human disease models III** Session Chairs: Gary Churchill and Elena de la Casa Esperon	Auditorio

09.15 O-27: Mouse *Slc9a8* Mutants Exhibit Retinal Degeneration Due to the Misregulation of Endosomal pH
Ian Jackson, MRC Human Genetics Unit, Edinburgh, UK

09.30 O-28: SEC23B Deficient Humans and Mice Exhibit Different Phenotypes
Rami Khoriaty, Internal Medicine, University of Michigan, Ann Arbor, MI

09.45 O-29: In Mice with Activated AKT, Constitutive Reduction of MTOR Reduces the Expression of *Cdk6* and Delays the Development of Thymic Lymphomas
Beverly Mock, National Cancer Institute/NIH, Bethesda, MD

10.00 O-30: A Suppressor Screen in Mouse *Mecp2* Identifies Cholesterol Homeostasis as a Therapeutic Target in Rett Syndrome
Monica Justice, Baylor College of Medicine, Houston, TX

10.15 O-31: Potent Modifiers of Intestinal Polyposis in Closely Related Inbred Mouse Strains
Linda Siracusa, Thomas Jefferson University, Philadelphia, PA

10.30 O-32: *CDCA7L* is a Male-Specific Susceptibility Locus and Oncogene in Glioma
Karlyne Reilly, National Cancer Institute/NIH, Bethesda, MD

10.45 – 11.30	Break	Hall Auditorio
11.30 – 12.45	**Advances in genome manipulation** Session Chairs: Jesus Perez-Losada and Lisa Tarantino	Auditorio

11.30 O-33: Rapid Conversion of EUCOMM / KOMP-CSD Alleles in Mouse Embryos

Using a Cell-Permeable Cre Recombinase
Edward Ryder, Wellcome Trust Sanger Institute, Hinxton, UK

11.45 O-34: Targeted Chromosomal Inactivation of the Mouse *Tyr* Insulators with Engineered Nucleases
Davide Seruggia, National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

12.00 O-35: Functional Deciphering of Genomic Boundaries Associated to Cellular Biological Requirements
Cristina Vicente-Garcia, National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

12.15 O-36: Examination of the Effect of a DNA Repair Defect on the Efficiency of ENU Mutagenesis
Jabier Gallego, Seattle Children's Research Institute, Seattle, WA

12.30 TBA
Student Presentation

12.45 – 14.15	Lunch (Cloisters), Exhibition & Posters	Hall Auditorio
12.45 – 14.15	Lunchtime Secretariat Meeting	Library
14.15 – 15.15	IMGS Business Meeting	Auditorio
15.15 – 16.30	**Stem cells and Development** Session Chairs: Simon Foote and Rami Khoriaty	Auditorio

15.15 O-37: Suppression and Quantitative Control of Meiotic Recombination Hotspot Activity
Pavlina Petkova, The Jackson Laboratory, Bar Harbor, ME

15.30 O-38: *Prdm9* Variability and its Effect on Genetic Recombination in a Robertsonian House Mouse Natural Population
Aurora Ruiz-Herrera, Universitat Autnoma de Barcelona, Barcelona, Spain

15.45 O-39: PRDM9-dependent modification organizes hotspot chromatin structure
Christopher Baker, The Jackson Laboratory, Bar Harbor, ME

16.00 O-40: Defects in *Nek8* Result in Abnormal Specification of Developmental Patterning, Polycystic Kidney Disease, and Impaired Response to Replication Stress
David Beier, Seattle Children's Research Institute, Seattle, WA

16.15 O-41: The ENU Induced Mutation, Germ Cell Depletion 2 (*gcd2*), is a Missense Mutation in *Kif18a* that Causes Cell-Type-Specific Mitotic Defects
Laura Reinholdt, The Jackson Laboratory, Bar Harbor, ME

16.30 – 17.00	Break	Hall Auditorio
17.00 – 18.45	**Large-scale resources** Session Chairs: Beverly Mock and Klaus Schughart	Auditorio

17.00 O-42: Rat Resource and Research Center
Elizabeth Bryda, University of Missouri-Columbia, Columbia, MO

17.15 O-43: Status of The Diversity Outbred Population
Gary Churchill, The Jackson Laboratory, Bar Harbor, ME

17.30 O-44: The Mouse Genomes Project: From Sequence Variation to Complete Genomes
Thomas Keane, Wellcome Trust Sanger Institute, Cambridge, UK

17.45 O-45: The Next-Gen Mouse and the Missense Mutation Library Delivering New Resources for Understanding Human Disease
Michael Dobbie, The Australian Phenomics Facility, The Australian Phenomics Network, The Australian National University, Canberra, Australia

18.00 O-46: Informatics for the International Mouse Phenotyping Consortium
Hugh Morgan, MRC Harwell, Harwell, UK

18.15 O-47: Quantitative Trait Locus Mapping in Diversity Outbred Mice
Daniel Gatti, The Jackson Laboratory, Bar Harbor, ME

18.30 O-48: KOMPute: Computational Prediction of Gene Function and Phenotypes for KOMP2
Carol Bult, The Jackson Laboratory, Bar Harbor, ME

20.00 –	Conference Dinner— buses depart at 20.00 from Colegio Fonseca	Castillo del Buen Amor

Posters:

P-1 **_Oune_, a Missense Mutation in _Tbx6_, Causes Congenital Vertebral Malformations in the Rat**
Koichiro Abe

SO-5/P-2 **Defining the Role of Mouse COPII Component SEC24C**
Elizabeth Adams

P-3 **The _Pirc_ Rat as a Model of Stochastic and Programmed Epigenetic Regulation**
James Amos-Landgraf

SO-7/P-4 **A Novel Role for an Olfactory Receptor in Pheromone Communication**
Andrew D Bard

P-5 **Genetic Basis of Host Susceptibility to Rift Valley Fever in Mice**
Leandro Batista

P-6 **Design of Validation Experiments Using the Collaborative Cross: A QTL for Sperm Curvilinear Velocity on Mouse Chromosome 2**
Timothy A Bell

P-7 **A Novel Intronic SNP in the Myosin Heavy Polypeptide 4 Gene is Responsible for the Mini-Muscle Phenotype Characterized by Major Reduction in Hindlimb Muscle Mass**
Timothy A Bell

P-8 **Interspecific Recombination Between Orthologous Human and Mouse BAC Clones in _E. coli_: Exploring Scalable Humanization of Cancer-Relevant Genes in the Mouse Genome**
David Bergstrom

P-9 **The Mouse Mutant Resource: Genetic, Genomic and Phenotypic Characterization of Spontaneous Mutant Mice Arising at The Jackson Laboratory**
David Bergstrom

P-10 **IMPReSS - International Mouse Phenotyping Resource of Standardized Screens**
Andrew Blake

SO-10/P-11 **Identification of Tumor Genetic Determinants of Response to Anthracyclines and Taxanes in an _Erbb2_ Breast Cancer Mouse Model**
Adrian Blanco-Gomez

P-12 **A Large-Scale Mouse Mutagenesis Screen for Identifying Models of Age-Related Musculoskeletal Disease**
Andrew Blease

P-13 **A Mutation in _Atp7a_ That is Sensitized in the Absence of Alpha Synuclein**
Deborah E Cabin

SO-2/P-14 **X Chromosome Inactivation Choice: Beyond the X Chromosome Controlling Element**
John D Calaway

P-15 ***Snai2* Participates In Different Stages Of Breast Cancer Evolution Through Its Role In Stem Cell Biology**
Sonia Castillo-Lluva

P-16 **Long-Range PCR and Next-Generation Sequecing to Identify an ENU Induced Mutation**
Yung-Hao Ching

P-17 **A Mutation in _Scn4a_ Modifies Huntington's Disease in Mice: A Potential Novel Link Between Huntington's Disease and SCN4A Channelopathies**
Silvia Corrochano

P-18 **Germline Variation Modulates Susceptibility to Aggressive Disease Development in a Mouse Model of Prostate Tumorigenesis**
Nigel Crawford

P-19 **ENU Mutagenesis Identifies Signalling Pathways**
Sally H Cross

P-20 **Annotating Non-Coding Genes in Mouse: How Conserved Are These Genes?**
Gloria Despacio-Reyes

P-22 **CrePortal.org, A Critical Resource for Conditional Mutagenesis**
Janan T Eppig

SO-11/P-23 ***Lman1* Deficient Mice Reveal New Insights Into Coagulation Factor V and VIII Secretion**
Lesley Everett

P-24 **Host Genetic Contributions Underlying Respiratory Function and Distress Following Acute Viral Infection.**
Martin T Ferris

P-25 **A Multi-allelic Clustering Approach for Improving Genotype Calling**
Chen-Ping Fu

SO-8/P-26 **Inferring Founder Probabilities in Admixed Animals using Genotyping Intensities**
Chen-Ping Fu

P-27 **Phenotypic Characterization of Mutant Mouse Lines in the German Mouse Clinic**
Helmut Fuchs

P-28 **Functionality of Amino Acid Substitutions and Other Single Nucleotide Variations in the Mouse**
Ryutaro Fukumura

SO-12/P-29 **Systems Pharmacogenomics Approach Identifies Synergistic Molecular Action of Combined MTOR/HDAC Inhibition on MYC**
Benjamin Gamache

P-30 **Mousebook – An Integrated Search Gateway for MRC Harwell's Aging Screen**
S Greenaway

P-31 **The European Mouse Mutant Archive - EMMA**
Michael Hagn

SO-1/P-32 **SINEUPs: Novel Antisense ncRNA Can Enhance Protein Translation**
Hazuki Takahashi

P-33 **The Metabolic Syndrome in Closely Related Berlin Fat Mouse Inbred Lines**
Sebastian Heise

P-34 **Introduction of a Human Epilepsy Mutation into the Mouse Sodium Channel Gene *Scn8a* Using TALEN Technology**
Julie M. Jones

SO-14/P-35 **Comparative mouse and human mRNA investigation suggest novel candidate genes involved in the pathogenesis of Major Depressive Disorder**
Irfan Jumabhoy

P-36 **Identification of Skin Tumor Modifier Genes in Japanese Wild Derived Inbred Mouse Strain, MSM/Ms.**
Okumura Kazuhiro

P-37 **A Nonsense Mutation in the Dystonin Gene Causes Hereditary Sensory and Autonomic Neuropathies in Mice**
Yoshiaki Kikkawa

SO-13/P-38 **Determining the Role of Innate Immunity in Mice Resistant to Rift Valley Fever**
Rashida Lathan

SO-9/P-39 **Analysis of the Host Response to Influenza A Virus Infection in the Collaborative Cross Founder Strains**
Sarah Leist

SO-15/P-40 **Exploring Regulatory Networks and Interactions of *Mir96* in the Developing Inner Ear**
Morag Lewis

P-41 **The Rare and Orphan Disease Center at The Jackson Laboratory**
Cathleen Lutz

P-42 **New Computational Method to Identify Epigenetics Markers for Colorectal Cancer Diagnosis**
H Mansour

P-43 **Nomenclature for Many-to-Many Homologene Sets**
Monica S McAndrews

P-44 **Generation of Knockdown Mice Using Pronuclear Injection-Based Targeted Transgenesis: Advantages and Limitations**
Hiromi Miura

P-45 **Compound Heterozygosity of the Functionally Null *Cdh23$^{v\text{-}ngt}$* and Hypomorphic *Cdh23^{ahl}* Alleles Leads to Early-Onset Progressive Hearing Loss in Mice**
Yuki Miyasaka

SO-4/P-46 **Starting at the Ends: High-Resolution Sex-Specific Linkage Maps of the Mouse Indicate that Recombination in Male Germline Progresses from Telomeres to Centromeres**
Andrew P Morgan

P-47 **The Collaborative Cross Mouse Population for Dissecting Host Susceptibility to Mixed Infection Inducing Alveolar Bone Loss**
Aysar Nashef

P-48 **Assessment of Comparative Functional Annotation Propagation in Mouse**
Li Ni

P-49 **Cardiac Phenotypes Arising From the Harwell Aging Screen**
Thomas Nicol

P-50 **Analysis of Directional Mutation Pressure in the Mouse Genome**
Satoshi Oota

P-51 **MegaMUGA, a Second-Generation Medium-Density Custom Designed Genotyping Array for the House Mouse**
Fernando Pardo-Manuel de Villena

P-52 **Generation of Pancreas CreER^{T2} Transgenic Mouse Lines for Time and Cell Specific Conditional Gene Inactivation**
Guillaume Pavlovic

P-53 **The Y Chromosome Histone Demethylase KDM5D Modifies Recombination Hotspot Activity**
Petko M Petkov

P-54 **Voluntary Exercise Reduces AOM-Induced Intestinal Tumor Number in a Strain-Dependent Manner**
Daniel Pomp

P-55 **Mapping Metabolic Traits in the Diversity Outbred Mouse Population**
Daniel Pomp

P-56 **Transgene Insertion Site Discovery by High-Throughput Sequencing of Mate Pair Libraries**
Laura Reinholdt

P-57 **Managing the Collaborative Cross: Effect of Housing Conditions on the Productivity of Large Breeding Programs**
Nashiya N Robinson

P-58 **Genomic Shuffling and Recombination: Implications for Mammalian Chromosomal Evolution**
Aurora Ruiz-Herrera

SO-6/P-59 **A Systems Biology Approach to Identify Interactions Between Breast Cancer and Ageing**
Maria del Mar Saez-Freire

P-60 **Nuclease-Mediated Gene Knockout in an Allele Refractory to Gene Targeting in ES Cells**
Thomas L. Saunders

P-61 **A Comparative Phenotypic Analysis of Two C57BL/6N Substrains**
Mohammed Selloum

P-62 **Productivity in the Collaborative Cross: Effect of Genetic Diversity on Mating Success, Litter Size, and Sex Ratio**
Ginger D Shaw

P-63 **Discovery and Interpretation of Phenomic Data from the Harwell Aging Screen**
Michelle Simon

P-64 **The FANTOM5 Project: Comprehensive Identification of Mammalian Promoters**
Harukazu Suzuki

P-65 **Novel QTLs Underlying Early-Onset, High-Frequency Hearing Loss in**

Abstracts:

Student and Post-Doc Presentations

SO-1/P-32: SINEUPs: Novel Antisense ncRNA Can Enhance Protein Translation

Hazuki Takahashi*[1], Silvia Zucchelli[2], Laura Cimatti[2], Francesca Fasolo[2], Aleks Schein[1], Ana Maria Suzuki[1], Alistair Forrest[1], Stefano Gustincich[2], and Piero Carninci[1]

[1]Division of Genomics Technologies RIKEN Center for Life Science technologies, Japan
[2]Area of Neuroscience International School for Advanced Studies, Italy

The mouse *Uchl1* (*PARK5*) RNA overlaps an antisense long non-coding RNA at its 5' end, in a so-called head to head orientation. In one familiar form of Parkinson's disease, UCHL1 protein is missing because of a mutation of *UCHL1* gene. We have recently found that *Uchl1os* RNA can regulate UCHL1 level by enhancing the translation efficiency of the overlapping sense mRNAs. This effect is mediated by the presence of a specific sequence, a short interspersed repetitive element B2 (SINE B2). Upon addition of rapamycin to mouse dopaminergic neuron cells (MN9D), *Uchl1os* shuttles from nucleus to cytoplasm then binds to 40S ribosome (Carrieri, C. et al. Nature. 2012 Nov 15;491(7424):454-7) enhancing translation. Interestingly, the *Uchl1os* does not affect *Uchl1* mRNA level. We named RNAs like the *Uchl1os* SINEUP, because they can up-regulate translation through a SINE element. SINEUP effect is also observed when sense and antisense RNA are co-transfected to cultured cells. Furthermore, SINEUPs designed to be antisense to enhanced green fluorescent protein (EGFP) mRNAs can upregulate EGFP protein expression, suggesting that artificial SINEUPs can be prepared by preparing mRNAs containing SINE B2 elements that overlap the mRNAs for which more protein is desired. The FANTOM consortium published earlier a large data set of mouse full-length cDNAs. Analysis of mouse antisense non-coding RNA containing SINE B2 sequence from the FANTOM database has identified a number of novel natural SINEUPs, which are also effective to enhance protein translation of their counterpart mRNAs, expanding the class of these RNAs. We are currently

mapping the active domains of these RNAs and are minimizing their size, to broadly expand their utilization. Besides being useful for protein production, SINEUPs could be a promising tool to perturb protein function by naturally enhancing protein activity, opposite to the effect of the miRNAs.

SO-2/P-14: X Chromosome Inactivation Choice: Beyond the X Chromosome Controlling Element

John D Calaway[*1,2,3,4,6], Alan B Lenarcic[1,6], John P Didion[1,2,6], Jeremy R Wang[5,6], Jeremy B Searle[7], Leonard McMillan[5,6], Daniel Pomp[1,2,6], William Valdar[1,2,6], and Fernando Pardo-Manuel de Villena[1,2,3,4,6]

[1]Department of Genetics
[2]Lineberger Comprehensive Cancer Center
[3]Carolina Center for Genome Sciences
[4]Curriculum in Genetics and Molecular Biology
[5]Department of Computer Science
[6]University of North Carolina, Chapel Hill, NC 27599
[7]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY 14853

X-chromosome inactivation (XCI) is the mammalian mechanism of dosage compensation that equalizes X-linked gene expression between the sexes. During early female embryogenesis, cells of the developing blastocyst independently choose to transcriptionally inactivate one of the two parental X Chromosomes. In mouse, primary XCI choice is influenced by the genotype of a *cis*-acting locus, the X chromosome controlling element (*Xce*). We have recently mapped *Xce* to a 176 kb region located 500 kb proximal to *Xist*. This region contains four tandem duplications and an inversion in C57BL/6J. We designed microarray probes to target this region and found that hybridization intensities perfectly partitions inbred and wild-derived mouse strains according to their *Xce* functional alleles. Furthermore, this approach predicted the functional *Xce* alleles in five uncharacterized wild-derived strains. We have proposed that structural variation at the *Xce* locus explains the presence of multiple functional alleles in *Mus*. To test this hypothesis and gain understanding of the molecular mechanism by which *Xce* acts during early XCI, we sequenced, at high depth (PacBio), bacterial artificial chromosome (BAC) clones spanning the *Xce* candidate interval. The BAC clones were derived from four inbred strains that carry three functionally different *Xce* alleles, C57BL/6J (Xce^b), C57BL/6NJ (Xce^b), C3H/HeJ (Xce^a), and MsM/Ms (Xce^e). Finally, our ongoing studies of XCI demonstrate that skewing is also controlled, in *cis* and in a parent-of-origin manner, by another X-linked locus and at least one autosomal modifier. We aim to use two genetically diverse mouse resources, the Collaborative Cross and the Diversity Outbred to identify these loci.

SO-3/P-68: Comparison of Meiotic Recombination Rates Using Immunostaining and Chromosomal Painting

Courtney Vaughn[*], Harry Sedgwick, Nadia Singh, and David Threadgill

North Carolina State University

Differences in the combination of alleles carried by individuals, which is generated by meiotic recombination, is important in influencing differences in disease susceptibility. We hypothesize that genetic differences among individuals control the number and distribution of meiotic recombination. To achieve this goal, we developed a new assay that combines analysis of meiotic recombination number and location with identification of individual chromosomes on which the recombination is occurring. Immunohistochemistry is used to observe two proteins; the first protein, mutL homolog 1, aggregates at sites of meiotic recombination on chromosomes of spermatocytes. Additionally, mutations in the *Mlh1* gene have been shown to strongly correlate to colorectal cancer. The second protein, synaptonemal complex protein 3, marks paired chromosomes. In situ hybridization with chromosome-specific paints marks individual chromosomes. We were able to optimize the process of imaging the proteins. Through this study, we will be able to better understand differences in how genetic variation is generated between individuals and support future studies investigating how variation in recombination is linked to differences in disease susceptibility, like that which occurs for cancer.

SO-4/P-46: Starting at the Ends: High-Resolution Sex-Specific Linkage Maps of the Mouse Indicate that Recombination in Male Germline Progresses from Telomeres to Centromeres

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Since the publication of the first comprehensive linkage map for the laboratory mouse, the architecture of recombination as a basic biological process has become amenable to investigation in mammalian model organisms. Here we take advantage of high-density genotyping and the unique pedigree structure of the incipient

Collaborative Cross to investigate the roles of sex and genetic background in mammalian recombination. Our results confirm the observation that map length is longer when measured through female meiosis than through male meiosis, but we find that this difference is modified by genotype at loci on both the X Chromosome and the autosomes. In addition, we report a striking concentration of recombination events in the distal ends of autosomes in male meiosis that is absent in female meiosis. The presence of this pattern in double-recombinant chromosomes, combined with the absence of a corresponding asymmetry in the distribution of double-strand breaks, indicates a temporally-regulated sequence of events in male meiosis in which the most distal crossover occurs first. This pattern is consistent with the timing of chromosome pairing and evolutionary constraints on male recombination. Finally, we identify large regions of reduced recombination frequency that together encompass 5% of the genome. Many of these “cold regions” are paradoxically enriched for segmental duplications, suggesting an inverse local correlation between recombination rate and mutation rate for large copy number variants. Our conclusions provide new insights into the mechanics and evolution of recombination as well as the design and interpretation of mapping experiments in mouse and human.

SO-5/P-2: Defining the Role of Mouse COPII Component SEC24C

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SEC24 is the COPII component thought to be primarily responsible for the recruitment of transmembrane cargoes or cargo adaptors into newly forming COPII vesicles on the ER membrane. Mammalian genomes encode four *Sec24* paralogs (*Sec24a-d)*, though little is known about their comparative functions and cargo-specificities. Based on protein sequence, SEC24A/B are more closely related to one another than they are to SEC24C/D. *Sec24b*^{Y613X} and *Sec24d*$^{Gt(RRR785)Byg}$ mice exhibit embryonic lethality, while *Sec24a*$^{tm1b(KOMP)Wtsi}$ knockouts have low cholesterol levels due to reduced secretion of PCSK9. All four paralogs are broadly expressed, and co-immunoprecipitations indicate that all four SEC24 paralogs interact with both paralogs of the SEC24 binding partner SEC23 (SEC23A/B) at relatively equivalent ratios.

To address the *in vivo* function of SEC24C, the allele *Sec24c*$^{tm1a(EUCOMM)Wtsi}$ was used to create mice deficient in SEC24C. This allele carries a gene-trap cassette insertion in intron 2, as well as loxP sites around exon 3. Removal of exon 3 by Cre creates a null allele. Mice deficient for SEC24C in all tissues exhibit embryonic lethality during mid-embryogenesis. Tissue-specific knockouts of *Sec24c* were generated using various cre lines; mice deficient for SEC24C in the liver, intestine, pancreas, or in smooth muscle are viable. Evidence for at least partial Cre-mediated excision in the target tissues, together with normal histology, demonstrate that SEC24C is not required for maintenance of these tissue types.

It is evident from our experiments that SEC24C and SEC24D are critical for survival. To test the potential overlap in function between SEC24C/D, we generated ES cells carrying the *Sec24c*$^{tm1(Sec24d)Dgi}$ allele, in which the SEC24C coding sequence has been largely replaced with SEC24D. Crossing mice with the *Sec24c*$^{tm1(Sec24d)Dgi}$ allele to either *Sec24c*$^{tm1d(EUCOMM)Wtsi}$ or *Sec24d*$^{Gt(RRR785)Byg}$ mice will test whether SEC24D can functionally replace SEC24C when its expression is driven by *Sec24c* regulatory elements.

SO-6/P-59: A Systems Biology Approach to Identify Interactions Between Breast Cancer and Ageing

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Breast cancer is a complex disease that results from the interaction of environmental factors with a number of genes with weak effects that explains the diverse cancer susceptibility and evolution among individuals. Ageing is the main epidemiological factor associated to an increased risk of breast cancer.

Our main goal was to identify the genetic and molecular determinants simultaneously associated with ageing and breast cancer susceptibility and evolution variability. Thus, we analysed a cohort of mice from a backcross

between a resistant strain to breast cancer (C57BL/6) and a susceptible one (FVB) that overexpresses the *Erbb2* (cNeu) proto-oncogene under the control of the Mammary Mouse Tumor Virus (MMTV) promoter.

We dissected the disease into different subphenotypes, such as tumor latency, local progression, number of tumors, metastasis development, duration of the disease and survival. A number of parameters associated to both ageing and cancer susceptibility and evolution, including oxidative stress (DNA, protein and lipid damage biomarkers), antioxidant defense system, pro-oxidant metabolic pathways and telomere length was used to quantify the grade of ageing in each individual. A multivariate approach was carried out to identify the complex interactions that exist between tumor and ageing subphenotypes.

To identify QTLs (Quantitative Trait Loci) associated with tumor and ageing subphenotypes, the cohort of mice was genotyped by SNP array and linkage analysis was performed using the R/QTL package. We identified a number of loci linked to both tumor and ageing parameters concomitantly.

The identification of the complex dynamic interactions between breast cancer and ageing subphenotypes, and the genetic determinants that regulate them will allow us to better understand the complex gene regulatory, metabolic and signaling networks involved. This eventually will lead us to improve the stratification of patients and the development of preventive and therapeutic strategies.

SO-7/P-4: A Novel Role for an Olfactory Receptor in Pheromone Communication

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Mice recognise and discriminate between small volatile odour molecules using an array of approximately 1000 genes encoding olfactory receptors (ORs), expressed in olfactory sensory neurons in the main olfactory epithelium (MOE). In contrast pheromone signals are detected by, and associated innate behaviours mediated by, the neurons of the physiologically distinct vomeronasal organ (VNO) which is specialised for this purpose with its own set of over 350 receptors (VRs). Studying OR and VR genes have historically proven challenging due to their high homology, large number and highly restricted cellular expression patterns.

However, using RNA sequencing, qPCR and in situ hybridization we have been able to identify two unusual OR genes that are specifically and atypically expressed in a small number of neurons in the mouse VNO. We focused on one of these, *Olfr692*, that is expressed in cells located basally in the VNO at a greater abundance than the entire MOE. These neurons co-express genes that are known to function in the VR transduction pathway but do not express genes involved in OR transduction, suggesting *Olfr692* may have been co-opted as a pheromone receptor or co-receptor. An analysis of its genomic and phylogenetic context, and an expression time series, lends support to this theory.

To identify ligands that specifically activate OLFR692, we have developed both in vivo and in vitro approaches to screen over 70 candidate odour molecules and range of complex sources of pheromones from animal secretions. I will present our progress on this screen and describe our ongoing effort to generate new mutant mouse resources to functionally interrogate this atypical olfactory receptor.

SO-8/P-26: Inferring Founder Probabilities in Admixed Animals using Genotyping Intensities

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We have trained and stored probabilistic genotype cluster models for each of the 8 founders of the Collaborative Cross/Diversity Outcross populations, and their F1 crosses on the new 78K-marker MegaMUGA genotyping array. These models provide a statistical framework from which we can infer the genotype probabilities at each marker, implicitly accounting for multiallelic SNPs and enabling more accurate and detailed analysis when compared to traditional biallelic genotypes. Using these clusters, we developed methods to infer most likely founder assignments and founder probabilities for admixed animals with haplotypes originating from the CC/DO founders, such as animals in the CC or DO populations. We compare two separate approaches: one which minimizes the overall distance between the target genome's xy intensities and the assigned founder clusters, and one which views the target genome as a hidden Markov model (HMM) and uses the forward-backward algorithm to find founder probabilities at each marker. We have sequenced 3 CC lines and found both approaches largely consistent with results from our sequencing data. In addition, by providing founder probabilities beyond the most likely founder at each marker, the HMM solved by the forward-backward algorithm provides insight into regions where multiple founders are identical by state. Its output can also be easily incorporated into QTL mapping software, effectively transforming genotype intensities into candidate gene regions.

SO-9/P-39: Analysis of the Host Response to Influenza A Virus Infection in the Collaborative Cross Founder Strains

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Influenza A virus poses a major health threat and caused multiple severe pandemics in recent human history with millions of deaths. The course and outcome of an influenza A infection is influenced by viral and 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. The main effect was determined by the *Mx1* allele but we also observed modifier effects of the genetic background.

SO-10/P-11: Identification of Tumor Genetic Determinants of Response to Anthracyclines and Taxanes in an *Erbb2* Breast Cancer Mouse Model

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An essential aspect of breast cancer is the different evolution among patients with the same histopathological disease. In addition, cancer is a tissue that grows in the context of the global organism, so it is possible to define two sources of variability in the behavior of the disease: intrinsic factors of tumor cells and extrinsic ones encompassing local and systemic factors.

Our aim was to identify tumor intrinsic factors responsible for the different response to chemotherapy with two drugs usually used to treat human breast cancer, Doxorubicin and Docetaxel.

For this purpose, we have collected tumors developed in a cohort of genetically heterogeneous mice from a backcross between a resistant strain (C57BL/6J) and a susceptible one (FVB/N-Tg(MMTVneu)202Mul/J) which overexpress the *Erbb2* (*Neu*) protooncogene under the control of Mouse Mammary Tumor Virus (MMTV) promoter. Backcross (Bx) mice were genotyped by SNP analysis (Illumina, N=377). We transplanted 125 Bx tumors into singenic B6FVBF1-Tg(MMTVneu) recipient mice to remove variability coming from host influences. Each tumor was transplanted into two recipient mice; each one of them was treated with a different drug. We classified tumors in terms of response to treatment. Linkage analysis was used to identify QTLs (Quantitative Trait Loci) controlling susceptibility to mammary cancer, evolution of the disease and response to therapy. We are in the process of refining those QTLs by the analysis of whole genome expression arrays (Affymetrix), before and after treatment. We are also studying molecular parameters and signaling pathways associated to tumor evolution. Identification of breast cancer modifier genes to chemotherapy response would allow us to learn about the mechanisms involved in partial success of actual chemotherapies, and eventually permit to develop more individualized preventive and therapeutic strategies.

SO-11/P-23: *Lman1* Deficient Mice Reveal New Insights Into Coagulation Factor V and VIII Secretion

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tm1a(KOMP)WtsiCombined coagulation factor V and VIII deficiency is an autosomal recessive disease characterized by ~90% reduction of factor V (F5, a.k.a. FV) and VIII (F8, a.k.a. FVIII), caused by mutations in *LMAN1* or *MCFD2*. LMAN1 and MCFD2 form a cargo receptor/adaptor complex that facilitates FV/FVIII secretion. We previously reported that C57BL/6-*Lman1*$^{Gt(XST010)Byg}$/*Lman1*$^{Gt(XST010)Byg}$ mice exhibit higher FV/FVII levels (~50% of wildtype) than in human patients, suggesting that *Lman1*$^{Gt(XST010)Byg}$ is hypomorphic. Additionally, a partial perinatal lethality of *Lman1*$^{Gt(XST010)Byg}$/*Lman1*$^{Gt(XST010)Byg}$ mice in specific genetic backgrounds suggests a broader role for LMAN1 in protein secretion. Since *Lman1*$^{Gt(XST010)Byg}$/*Lman1*$^{Gt(XST010)Byg}$ mice retain ~1% of normal *Lman1* expression, two other modified alleles were generated. The conditional allele (*Lman1*$^{tm1a(KOMP)Wtsi}$) does not result in lethality and C57BL/6J-*Lman1*$^{tm1a(KOMP)Wtsi}$/*Lman1*$^{tm1a(KOMP)Wtsi}$ mice have ~8% of normal *Lman1* expression. C57BL/6J-*Lman1*$^{tm1a(KOMP)Wtsi}$/*Lman1*$^{tm1a(KOMP)Wtsi}$ mice are also viable in preliminary studies and completely LMAN1 deficient, indicating that the *Lman1*$^{Gt(XST010)Byg}$/*Lman1*$^{Gt(XST010)Byg}$ lethality may have resulted from a passenger-gene effect. To characterize the relationship between *Lman1* expression and LMAN1-cargo secretion, FV/FVIII activities were measured in these mice. *Lman1*$^{tm1a(KOMP)Wtsi}$/*Lman1*$^{tm1a(KOMP)Wtsi}$ mice exhibit ~80% of normal FV and FVIII levels relative to wildtype ($p<0.001$), compared to ~50% levels in *Lman1*$^{Gt(XST010)Byg}$/*LMAN1*$^{Gt(XST010)Byg}$ ($p<1.0x10^{-4}$) and *Lman1*$^{tm1d(KOMP)Wtsi}$/*Lman1*$^{tm1d(KOMP)Wtsi}$ mice ($p<0.005$). Whereas it is well known that murine FV is produced in megakaryocytes and hepatocytes, the cellular source for FVIII biosynthesis is controversial, having either an endothelial or hepatocyte origin. We therefore crossed mice carrying the *Lman1*$^{tm1c(KOMP)Wsti}$ conditional allele to B6.Cg-Tg(Tek-cre)12Flv/J (endothelium and hematopoietic tissues) or B6.Cg-Tg(Alb-cre)21Mgn/J (hepatocytes) transgenic mice to generate tissue-specific knockout mice. The endothelium-specific knockout mice exhibit normal FV levels, but significantly reduced FVIII levels (52.5%) relative to wildtype mice ($p< 3.0x10^{-6}$). Similarly, hepatocyte-specific knockout mice exhibit normal FVIII levels, but low FV levels (33.5%) relative to wildtype mice ($p<4.0x10^{-7}$). These results are consistent with the hypothesis that endothelial cells are the primary site of FVIII biosynthesis *in vivo*.

SO-12/P-29: Systems Pharmacogenomics Approach Identifies Synergistic Molecular Action of Combined MTOR/HDAC Inhibition on MYC

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Multiple myeloma (MM) and murine plasmacytoma (PCT) are rare mature B-lymphoid malignancies. Allelic variants of *Mtor* and *Cdkn2a* affect susceptibility to PCT, and functional alterations in the PI3K/MTOR and CYCLIN/CDK/CDKI/RB (RB) pathways are common to both malignancies. We found that combining sirolimus (rapamycin), an inhibitor of mechanistic target of rapamycin (MTOR), with entinostat (MS-275), a selective class I histone deacytlase (HDAC) inhibitor, was synergistic in controlling 90% of tested cell lines derived from B cell malignancies *in vitro,* effective in limiting xenograft growth *in vivo*, and diminished cellular viability in *ex vivo* patient samples. Similarly, the combination reduced tumor burden and volume and increased survival in a long-term, *in-vivo* study in C.B6-*Bcl2l1* mice. To examine the core synergistic consequence of combining entinostat with sirolimus, an integrated, systems-level approach was used. Weighted gene co-expression analysis (WGCNA) of GEP data from MM cells treated individually and in combination was used to identify a distinct module of 126 genes cooperatively affected by both drugs. Of the cooperatively affected genes, 37 were found to be differentially expressed in MM and predictive of survival ($p<0.01$). Ingenuity upstream analysis identified MYC as a potential core regulator of the synergistic transcriptional response. MYC protein, but not mRNA, decreased in response to the drug combination when examined by Western blot and NanoString analyses, respectively. Using tet-off, MYC-inducible transformed P493 cells, the necessity of MYC for the drop in cellular viability and response of the gene signature to the combination was evident. Furthermore, the drug combination elicited greater rates of MYC protein degradation upon treatment of MM cells with cycloheximide. Results have shown the drug combination affects both MYC protein synthesis and degradation, although further studies are needed to delineate the contribution of each drug individually.

SO-13/P-38: Determining the Role of Innate Immunity in Mice Resistant to Rift Valley Fever

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Introduction: Rift Valley Fever virus (RVFV) is an arbovirus prevalent in Africa and the Middle East. RVFV causes a 100% effective abortion rate among infected livestock and is characterized by hepatitis with hemorrhage in ~3% of infected humans. We previously identified a host genetic basis for RVFV resistance in BALB/cByJ mice in comparison to moderately susceptible C57BL/6 mice and highly susceptible, wild-derived MBT/Pas mice after experimental infection with RVFV (ZH548 strain), and have developed congenics that isolate BALB/cByJ resistant loci and MBT/Pas susceptible loci. Objective: Since innate immunity plays a crucial step in limiting viral replication during RVFV infection, the potential role of host immune response as a cellular mechanism of resistance was investigated. Materials and Method: Non-infected and infected mice of the three strains, were characterized through flow cytometric analysis. Cell suspensions derived from the brain, liver, spleen were labeled to identify dendritic cells, macrophages, B-cells, neutrophils, natural killer (NK) cells, T-cells, and to identify cells infected by virus. Results: Results indicate lower basal levels of NK cells and neutrophils among susceptible MBT/Pas mice. These low levels are maintained post RVFV infection. Conclusion: Deficiency in NK

cells is a cause of human susceptibility to some viruses, such as Herpes Simplex. Lower levels of this cell type in MBT/Pas mice suggest a NK-deficient mechanism for RVFV susceptibility, and can serve as a biological platform for gene discovery.

SO-14/P-35: Comparative mouse and human mRNA investigation suggest novel candidate genes involved in the pathogenesis of Major Depressive Disorder

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Aim: To identify novel genes associated with MDD and pharmacological treatment response using animal and human mRNA studies.

Methods and Materials: Weighted Gene Network Co-Expression analysis (WGCNA) was used to uncover genes associated with stress factors in mouse and to inform mRNA probeset selection in a post-mortem study on depression.

Results: 171 genes were found to be differentially regulated in response to both early and late stress protocols in a mouse study. Nine human genes, orthologous to mouse genes differentially expressed by stress, were also found to be dysregulated in depressed cases in a human post-mortem brain study from the Stanley Brain Consortium.

Conclusion: Several novel genes associated with depression were uncovered, including *NOVA1* and *USP9X*. Moreover, we found further evidence in support for hippocampal neurogenesis and peripheral inflammation in MDD.

SO-15/P-40: Exploring Regulatory Networks and Interactions of *Mir96* in the Developing Inner Ear

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Mutations in the microRNA *Mir96* cause deafness in mice and humans. In the *diminuendo* mouse, which carries a single base pair change in the seed region of *Mir96*, the sensory hair cells crucial for hearing fail to develop fully and retain immature characteristics, suggesting that *Mir96* plays an important role in coordinating hair cell maturation. Microarrays we have carried out show that many genes are dysregulated in the *diminuendo* inner ear, including several known to be important for hearing. However, the pathways through which *Mir96* acts are unknown. We have chosen several complementary approaches to identify potential networks of *Mir96* using this transcriptional data.

Firstly, focussing on individual interactions, we constructed a network using a database of regulatory interactions manually curated from the literature. This network connects *Mir96* to as many of the dysregulated genes as possible. It is directional and internally consistent, and we have confirmed some of its predictions. We then took a pathway-based approach using the InnateDB database to find interactors of potential *Mir96* targets identified from the literature and our data. The resulting interactome is not a directional network but offers a wider view of pathways through which *Mir96* might be acting, via its direct targets.

Finally, we used gene set enrichment analysis to identify gene sets in which the dysregulated genes are enriched. This is distinct from the previous two approaches in that it does not rely on correctly identifying the direct targets of *Mir96*, and the gene sets are defined by the user. This approach does not result in a network or interactome, but instead identifies pathways implicated in the *diminuendo* phenotype. Because *Mir96* is a master regulator of hair cell maturation, understanding the pathways it regulates could lead to potential therapeutic targets for treating hearing loss in the wider population.

SO-16/P-66: A Sensitized Whole Genome ENU Mutagenesis Screen Identifies a Novel Suppressor Region for Lethal Thrombosis in the Factor V Leiden Mouse

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Only ~10% of individuals carrying the common venous thrombosis risk factor, Factor V Leiden (FVL) will develop venous thrombosis in their lifetime. In order to identify potential FVL modifier genes, we performed a sensitized dominant ENU mutagenesis screen, based on the perinatal synthetic lethal thrombosis previously observed in

mice homozygous for FVL ($F5^{tm2Dgi}/F5^{tm2Dgi}$) and hemizygous for tissue factor pathway inhibitor deficiency (*Tfpi*+/-).

ENU-treated male $F5^{L/L}$ mice were crossed with $F5^{tm2Dgi}/F5^{tm2Dgi}$ *Tfpi*+/- females (both C57BL/6J). Analysis of 7,128 G1 offspring (~2X genome coverage) identified 15 $F5^{tm2Dgi}/F5^{tm2Dgi}$ *Tfpi*+/- mice which successfully transmitted the putative rescue mutation to 2 or more G2 progeny. We applied whole exome sequencing on 8 mouse lines with the highest penetrance. We identified a nonsynonymous single nucleotide variant (SNV) in the *Actr2* gene which closely segregated with $F5^{tm2Dgi}/F5^{tm2Dgi}$ *Tfpi*+/- survival in one of these lines (15/16 re-sequenced $F5^{tm2Dgi}/F5^{tm2Dgi}$ *Tfpi*+/- progeny, $p<0.0001$). In another line, where no obvious exonic SNV was identified, we used the SNPs introduced by backcrossing to a second strain (129S1/Svlmj) to map the region co-segregating with the 'rescue' phenotype. Using whole exome sequencing data from 4 mice in the pedigree followed by individual SNV genotyping in additional mice, we identified a ~6Mb interval (Chr 3:108.9-115.3Mb) that is shared in 44 out of 53 surviving $F5^{tm2Dgi}/F5^{tm2Dgi}$ *Tfpi*+/- mice in the pedigree ($p=1.53x10^{-6}$), a region containing <15 annotated genes.

We are currently resequencing areas with lower coverage in the intial whole exome analysis to exlude any ENU induced SNVs that may have been missed. In addition, we are testing for allele specific expression differences in multiple tissues for all the genes in the candidate region.

Oral Presentations

O-1: Transposon Mutagenesis Uncovers Evolutionary Forces Driving BRAFV600E Melanoma

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Approximately 50% of melanomas harbor an activating (V600E) mutation in the *BRAF* proto-oncogene. Despite the development of highly successful therapies targeting BRAFV600E the overall survival in patients treated with these drugs is ~16 months. By performing a Sleeping Beauty [SB] transposon mutagenesis screen in mice carrying an inducible $Braf^{tm1Mmcm}$ (BRAFV600E) allele we have attempted to define the evolutionary forces driving BRAFV600E melanoma with the goal of identifying targets for drugs that might synergize with those targeting BRAFV600E. Analysis of the transposon insertion sites in 70 SB|BRAFV600E melanomas identified a discovery set of 1,232 statistically defined melanoma candidate cancer (CAN) genes. Many biological signaling pathways and cellular processes were enriched for these CAN genes, including Wnt/Beta-catenin, TGF-Beta, PI3K and MAPK signaling pathways, and biological processes regulating ubiquitin mediated proteolysis, tight junctions, cell cycle and axonal guidance. More than 500 CAN genes were also enriched for mutations in human melanoma sequencing data sets or showed significant clinical associations between RNA abundance and advanced stage metastatic melanoma patient survival. CAN genes were also more highly connected to one another than predicted by chance, indicating that transposon mutagenesis is selecting for mutations in proteins that interact. Transposon mutagenesis has thus helped to uncover the evolutionary forces driving BRAFV600E melanoma and identified a rich resource of genes with potential clinical importance to BRAFV600E melanoma.

O-2: A metastasis susceptibility gene for estrogen receptor negative breast cancer maps to the distal end of Chromosome 6

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Metastatic disease is the primary cause of patient mortality for solid tumors. Metastatic disease consists of dissemination of tumor cells from the primary lesion, transit through the body, arrest at a secondary site, followed by outgrowth in the new location. The process is highly inefficient and may take years or decades from initial dissemination to clinical disease. As a result the mechanisms responsible for progression to metastatic disease are poorly understood due to the inability to access and study tumor cells that will ultimately form secondary tumors.

To better understand this process our laboratory has used susceptibility screens to identify polymorphic genes that influence the development of metastases. Previously, by breeding F1 hybrids between inbred mouse strains and the highly metastatic *Tg(MMTV-PyVT)634Mul* (*MMTV-PyMT*) transgenic mouse model (FVB/NJ background) we demonstrated that genetic background had a significant impact on tumor latency, growth and metastatic disease. The MOLF/Ei genotype significantly delayed tumor diagnosis from 58 to 80 days after birth ($p < 0.008$), reduced tumor growth by 30% ($p < 0.005$) and reduced the number of pulmonary metastases ($p = 0.002$) and was selected for further study.

A backcross [(MOLF/Ei x MMTV-PyMT) x FVB/NJ] of 172 animals was generated and genotyped at CIDR using the 1449 SNP Illumina Mouse Medium Density Linkage Panel. Analysis using the J/QTL package revealed a single significant linkage peak at the distal end of Chromosome 6 for all three tumor phenotypes. eQTL analysis identified 7 genes within the 95% confidence interval that correlated with metastatic capacity. Preliminary modeling using tumor engrafting and gene trap mouse models indicate that at least two genes play a role in metastatic progression. Bioinformatics analysis is consistent with a role of these genes in human disease and indicates that it is specific for the poor prognosis estrogen receptor negative subclass. Further characterization of these genes is ongoing.

O-3: The role of *Far2* in the initiation of age-related kidney disease.

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Aging of the kidney is associated with renal damage, in particular mesangial matrix expansion (MME), a process in which the mesangial cells of the glomerulus produce more matrix proteins than are being degraded. To unravel the mechanisms of aging it is important to identify genes involved in this process, since this might help to design novel therapeutic modalities aimed at prevention and regression.

We recently used genome-wide association mapping in mouse inbred strains to determine genetic loci associated with the presence of MME at 20 months of age. This analysis identified a significant association with a 200-Kb haplotype block on Chromosome 6 containing *Far2*. Sequencing revealed that the strains with MME contain a 9-bp sequence in the 5′UTR of *Far2*, which is absent in most of the strains without MME. Real-time PCR showed a 2-fold increase in the expression of *Far2* in the kidneys of strains with the insert compared to strains without the insert. Subsequent gel shift assays and cloning of the two allelic forms of the 5′UTR of *Far2* into a luciferase reporter vector showed that this sequence difference causes a difference in transcription factor binding sites and leads to the difference in *Far2* expression. Overexpression of *Far2* in a mouse mesangial cell line induces upregulation of platelet activating factor, the fibrotic marker transforming growth factor β, and a number of renal damage-related genes. Labeling experiments show that FAR2 catalyzes the reduction of fatty acyl-CoA to fatty alcohols, which are then used as precursors of platelet activating factor.

This is a novel pathway involved in renal aging and our future efforts are aimed at inhibition of this pathway in order to slow down mesangial matrix expansion and the subsequent loss in renal function.

O-4: Dissection Of ERBB2 Breast Cancer Heterogeneity By A Systems Biology Approach

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Breast cancer shows different clinical evolution among patients who seemingly have the same hystopathologycal process. Clinical cancer evolution reflects perturbations of complex networks constituted by a number of links at different levels encompassing genomic, RNA, signalling pathways, metabolic physiological and clinical pathophenotypes. All these layers would be interconnected and followed a model of Systems Biology. To identify these interactions would help to understand the different behaviour of the disease among individuals, and improve strategies to prevent and treat breast cancer in a more personalized manner. Thus, we analyzed ERBB2 positive breast cancer behavior in a backcross population of mice. We dissected the disease in different clinical pathophenotypes and identified some of the intra and inter-level connections at genetically, RNA expression, cell signaling and metabolic steps that were associated with the heterogeneous susceptibility and evolution of the disease. The tumor RNA expression pattern identified in the backcross recapitulated the human one better than the pure FVB strain. Interestingly, not only tumor signaling pathways, but also liver ones showed strong associations with tumor behavior heterogeneity. Additionally, we also identified the genetic background influence

at all of these different molecular and pathophenotypic levels.

This strategy also permitted to identify particular features of the disease, for example those tumors that metastasized to the lung showed lower levels of pAKT1(ser473); and those with short latency showed a block in the phosphorylation at MAP3K (MEK) level. We also carried out a high throughput metabolic study in serum from disease-free mice at three months of age that permitted us to identify a number of metabolites that could predict different tumor traits evolution. As conclusion, we have used a Systems Biology approach to define the complex molecular interactions at genetically, transcriptional, cell signaling and metabolic levels that define the heterogeneous susceptibility and evolution of ERBB2 breast cancer.

O-5: Amino acid differences between mouse and human alpha synuclein influence pathogenicity in yeast and mice

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Synuclein, alpha (SNCA) is linked to rare familial forms of Parkinson's disease and to the common sporadic form. Human A53T mutant SNCA is associated with a familial form of Parkinson's, but in most vertebrates, the WT residue at position 53 is threonine. Besides residue 53, mouse and human SNCA differ by 6 of 140 amino acids. We hypothesized that one or more of these amino acid differences protects mice against the presence of pathogenic Thr53. We generated constructs in which human amino acids were individually substituted into mouse Snca and tested these variants in yeast. In a galactose-inducible expression system, using a low copy number vector, four of our mouse variants inhibited yeast growth. Two abolished growth as drastically as A53T human SNCA; human and mouse wild type proteins did not inhibit growth. We tested the two most cytotoxic SNCA variants in mammalian brain, along with mouse WT SNCA and human A53T SNCA. Purified SNCA proteins were fibrillized in vitro, and after sonication were injected into brains of prion promoter-regulated A53T SNCA cDNA transgenic mice Tg(Prnp-SNCA*A53T);*Snca*^{tm1nbm}. One mouse variant triggered a spinal cord synucleinopathy as quickly as did A53T human SNCA. The transgenic mice injected with misfolded/oligomerized WT mouse SNCA had a significantly longer lifespan, though they did develop the synucleinopathy. All had significantly shorter lifespans than control transgenics. Thus mouse SNCA and two mouse variants can misfold or oligomerize in vitro to form a pathogenic template that triggers propagation of pathology through the nervous system from the injection site in the midbrain to the spinal cord. The remaining variants are currently being tested. We hypothesize that differences in cytotoxicity in yeast may reflect differences in the propensity of different SNCA species to adopt a pathogenic conformation in vivo, and we have identified amino acids that may influence that propensity.

O-6: The Harwell Ageing Mutant Screen

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Diseases associated with ageing pose an increasing burden on society and represent a vital imperative for research in the biomedical sciences. We are undertaking the first large-scale project to investigate the interaction between genetic variation and the pleiotropic effects of ageing. We are employing ENU mutagenesis and phenotyping to specifically generate new models of late onset or age-related disease and identify genes or pathways associated with such diseases. Analysis occurs throughout the life of the mice and the phenotypes included in the screening are diabetes and metabolism, neurobehaviour, bone analysis, renal function, cardiac disease, liver function, sensorineural (vision and hearing), and a comprehensive clinical chemistry screening. To date we have approximately 150 G3 pedigrees that have entered the Ageing Screen and have identified phenotypes throughout the lifespan of the mice. Nearly 50% of the phenotypes were initially identified after 5 months of age and include; hypertrophic cardiac myopathy, osteoarthritis, obesity, presbycusis, and retinal degeneration. To facilitate rapid gene identification without recourse to further breeding and ageing, we are screening large pedigrees of ~100 mice followed by mapping and whole genome sequencing to identify causative loci. Multiple, segregating phenotypes are found in each pedigree with some containing up to 4 separate phenotypes

We are capitalising on the power of the whole genome sequencing by pre-emptively sequencing G1 founder males for a number of pedigrees firstly, generate a database of archived mutations that may be of interest to the scientific community, secondly, to allow the combination of gene-driven and phenotype-driven screens in a single pipeline by applying more detailed phenotyping of mice of specific genotypes, and finally, allow us to identify functional, non-coding mutations. We have mapped several mutants and are now investigating individual pathways and how they interact with the ageing process to result in a disease phenotype.

O-7: An Aging Screen Identifies Novel Models of Age-Related Hearing Loss

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Age-related hearing loss (ARHL) is a significant health and social burden on the population and is one of the four most common chronic health conditions experienced by the elderly. Greater than 25% of adults aged 50 and over have a hearing loss of 30 dB or more (increasing to 70-80% of people aged 75 and over). At MRC Harwell we are utilizing a large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis screen to identify mouse models of aging. G3 pedigrees, of ~100 mice, are bred and enter a phenotyping pipeline comprising recurrent assessment across a wide range of disease areas including, diabetes, metabolism, neurobehaviour, bone, renal, cardiac, and sensorineural. The Deafness Models and Mechanisms team is taking advantage of this screen to identify models of ARHL, employing recurrent Clickbox and Auditory-Evoked Brainstem Response phenotyping.

As of July 2013, 121 G3 pedigrees have entered the phenotyping pipeline, of which 95 have completed auditory screening. To date 17 pedigrees (~14%) have confirmed auditory phenotypes. Of these, 12 pedigrees display early-onset hearing loss as evidenced by elevated hearing thresholds by 3 months of age. The remaining 5 pedigrees exhibit late-onset progressive hearing loss with elevated hearing thresholds evident from 6 months onwards, which is most pronounced at the highest frequency tested (32kHz). Currently, 2 of the ARHL pedigrees (MPC-96 and MPC-151) have undergone genome-wide mapping and whole-genome sequencing (WGS), demonstrating that each maps to a distinct and novel deafness gene. Studies to relate mutant protein to phenotype are ongoing. Genome mapping and sequencing of the remaining 3 ARHL models (MPC-202, MPC-203 and MPC-205) is underway.

The Harwell Aging Mutant Screen is producing pedigrees with interesting age-related auditory phenotypes. Investigation of these, and as yet unidentified pedigrees, promises to increase our understanding of the genetics underlying hearing and its age-related decline.

O-8: The effect of dietary deficiencies during gestation on adult behavior and gene expression in mice

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Exposure to dietary deficiencies during gestation has been shown to result in an increased risk for affective disorders in human populations. *In utero* exposure to nutrient deficient diets has been modeled successfully in rats, but very few studies have examined genetically stable mouse lines, for which many more genomic analysis tools exist. In a pilot study, we tested the effect of gestational and postpartum low protein, low vitamin D and methyl deficiency on reciprocal F1 female offspring of C57BL/6J and NOD/ShiLtJ mice. Females were exposed to various diets for 5 weeks prior to mating and throughout pregnancy until weaning. Reciprocal F1 offspring were tested in a variety of behavioral assays to study the effects of gestational diet on anxiety and depression-related behaviors, sensorimotor gating, social interaction and locomotor response to psychostimulants. We observed significant reciprocal strain differences in novelty-induced locomotor activation and other behavioral measures. Diet-dependent, reciprocal cross differences were also observed for anxiety-related behaviors. Surprisingly, however, many behaviors in the F1 mice were largely unaffected by what would be considered severe dietary deficiencies *in utero*. Three days after behavioral testing, mice were euthanized, whole brain tissue collected and genome-wide gene expression was assessed using the Affymetrix Mouse Gene 1.0 ST Array. We identified transcripts that varied significantly depending on maternal strain (i.e. parent of origin effects). These included 4 known imprinted clusters on Chrs 7 (*Snord115, Snord116, Ndn*), 11 (*Zrsr1*), 12 (*Rian*) and 17 (*Airn*). In humans, *SNORD* genes and *NDN* are associated with Prader-Willi syndrome. An additional 26 transcripts showed significant parent of origin effects at loci for which imprinting has not yet been reported.

O-9: Multiple QTL mapping of cardiac collagen deposit in F2 population of *Scn5a* mutant mice reveals interaction between *Fgf1* and *Pdlim3*, *Gpr158* & *Itga6*

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With aging and in cardiac disease increasing fibrosis due to collagen deposition disturbs cell-cell coupling of cardiomyocytes, providing a substrate for arrhythmia. In this study we set out to identify genetic modifiers of collagen deposition in heart. We exploited the genetic variability among F2 progeny of 129P2 and FVBN/J mice carrying the $Scn5a^{tm1Care}$/+ mutation to identify genes that influence the amount of collagen deposition in left ventricular (LV) myocardium.

Relative amounts of collagen were determined in 65 F2-mice and combined with genome-wide genotypic and expression data to identify collagen-QTLs (cQTLs) and the underlying expression QTLs (eQTLs). In both collagen-QTL mapping as well as eQTL mapping we identified significant co-factors through multiple QTL mapping (MQM).

A significant cQTL was identified on mouse Chr 8 and after correction for cofactors using MQM an additional cQTL was found on mouse Chr 2. For both these loci a significant co-factor was identified on Chr 18. Of the 24 eQTLs mapping to the Chr 8-cQTL, 8 transcripts correlated to relative collagen amount. Similarly, of the 6 Chr 2-cQTL eQTLs only *Gpr158* (significantly) and of the 12 eQTLs of Chr 18-co-factor-region only *Fgf1* (suggestively) correlated with the relative collagen amount. Furthermore, two cQTL-eQTLs, *Pdlim3* (Chr 8) and *Itga6* (Chr 2), had a significant co-factor on Chr 18 that coincided with the Chr 18 collagen cofactor. We validated the interaction of FGF1 with these transcripts and collagen production in vitro in isolated cardiac fibroblasts. In conclusion, we mapped, for the first time, a genetic network that modulates collagen deposition in mouse LV myocardium.

O-10: Studying mouse knock-out lines for resistance and susceptibility to influenza A virus infection

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Every year, about 500 million people worldwide are infected with the influenza A virus, of which about 500,000 die, and the emergence of new influenza subtypes caused several severe pandemics in the past. Thus, a deeper understanding is needed of the complex interplay between host and pathogen during influenza A infections. The mouse has been proven to represent an excellent animal model to study host-pathogen-interactions during severe influenza infections. Studies on genetic factors in the mouse demonstrated that there is a genetic predisposition for severe morbidity and mortality in the mammalian organism and allowed the identification of first susceptibility genes in humans.

Here, we report on the host response of several mouse knock-out mutants carrying mutations in several genes, *e.g. Tmprss2, Tmprss4, Serpine1, Flt3, Plau, Ifit1, Ifi27, Mysm1, Reg3g, Rag2, Mmp7, Irf3, Socs3 and Tlr13* to infections with different influenza A subtypes. In some mutant lines, no phenotypic differences were observed, whereas most mutant lines revealed an increased susceptibility compared to the wild type C57BL/6J strain. Most remarkably, deletion of the *Tmprss2* gene completely protected mice from weight loss and death upon infection with influenza A viruses of the H1N1 subtype. Furthermore, lung pathology and viral spread was strongly reduced and mutant mice did not process the hemagglutinin protein.

O-11: Genetic Regulation of *Zfp30*, *Cxcl1*, and Neutrophilic Inflammation in Mouse Lung

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Allergic asthma is complex disease characterized in part by granulocytic inflammation of the airways. In addition to eosinophils, neutrophils (PMN) are also present, particularly in cases of severe asthma. We sought to identify the genetic determinants of neutrophilic inflammation in a mouse model of house dust mite (HDM)-induced asthma. We applied an HDM model of allergic asthma to the eight founder strains of the Collaborative Cross (CC) and 151 incipient lines of the CC ("preCC"). Lung lavage fluid was analyzed for PMN count and the concentration of CXCL1, a hallmark PMN chemokine. PMN and CXCL1 were strongly correlated in preCC mice. We used quantitative trait locus (QTL) mapping to identify three variants affecting PMN, one of which co-localized with a QTL for *Cxcl1* on Chromosome (Chr) 7. Additionally, we measured whole lung gene expression by microarray and used expression QTL mapping to implicate a variant in the gene *Zfp30* in the CXCL1/PMN response. This genetic variant regulates both CXCL1 and PMN by altering *Zfp30* expression, and we model the relationships between the QTL and these three endophenotypes. Furthermore, we demonstrate that *Zfp30* mRNA and protein is expressed in airway epithelia. Our results provide strong evidence that *Zfp30* is a novel candidate gene for neutrophilic airway inflammation.

O-12: QTL identification and confirmation of *Trim55* contribution to SARS-CoV-induced vascular cuffing using the Collaborative Cross

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SARS-CoV causes an atypical pneumonia that is fatal in 10% of all patients and 50% of elderly patients. Host genetic background is thought to impact the outcome of infection but this is difficult to determine in humans due to the limited nature of the SARS outbreak and inconsistent sample collection during the epidemic. Existing animal models of SARS-CoV infection have revealed that this lethal pulmonary infection causes a denuding bronchiolitis associated with acute respiratory failure. Aged mouse populations show lung injury similar to acute respiratory distress syndrome (ARDS) and organized inflammation consistent with diffuse alveolar damage (DAD), replicating a key phenotype of SARS infection in humans. Using genetically diverse incipient lines of Collaborative Cross mouse resource we have greatly expanded the range of phenotypes observed in response to SARS-CoV infection including weight loss and viral load in the lung. Young preCC mice showed mild to extreme lung damage, including development of hyaline membranes and ARDS like phenotypes previously only seen in aged animals infected with SARS-CoV. Genome scans revealed a locus responsible for vascular cuffing on Chromosome 3 as well as potential loci for control of virus load and recruitment of eosinophils to the lung. The Chromosome 3 QTL covered an 8.5 Mb region containing 23 genes and 13ncRNAs. Further analysis of this QTL showed that the phenotype was driven by the C57BL/6J and WSB parent alleles, enabling the likely QTL interval to be narrowed to a 500kb region containing the *Trim55* gene. *Trim55* has previously been identified as an E3 ubiquitin ligase with a role in muscle fiber formation. Transcriptomic data and lung pathology from *Trim55* knockout mice confirmed the identification of *Trim55* as a contributor to SARS-CoV-induced vascular cuffing, indicating a novel function for *Trim55*.

O-13: Impact of Changes in Microbiota Associated with Rederivation of a Mouse Model of Inflammatory Bowel Disease

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Rederivation of mice is a common practice used to render rodents free of unwanted infectious diseases. This also occurs when mutant rodent strains, such as those maintained by the Mutant Mouse Regional Resource Center (MMRRC) and Rat Resource and Research Center (RRRC), are cryopreserved and subsequently recovered for use in research. While this process eliminates unwanted pathogens, it may also result in changes in microbiota that impact model phenotypes. To this end, we investigated whether rederivation of a common mouse model of inflammatory bowel disease (IBD) onto different recipient strains of mice would result in changes in microbiota that correlated with changes in disease severity. In addition, we assessed whether the segmented filamentous bacterium (SFB) status of recipients impacts disease severity. C57BL/6 (B6) and C3H/HeJBir mice with targeted mutations in the *Il10* gene were rederived onto SFB-negative CD-1, SFB-negative B6, or SFB-positive B6 recipient mothers. To induce inflammatory bowel disease, pups were inoculated with *Helicobacter hepaticus* at 3 and 5 days post weaning. At 90 days post-inoculation, cecal and colonic lesion scores were evaluated using histopathology and changes in microbiota were assessed using Autosomal Ribosomal Intergenic Spacer Analysis (ARISA) and/or sequencing of the *16S* rRNA gene. Differences in the microbiota were seen between mice derived onto SFB-negative CD1 or B6 recipients; however no differences in IBD lesion scores were seen. Differences in the microbiota were also seen between mice derived onto SFB-negative and SFB-positive B6 mice. Moreover, mean cecal lesion scores were significantly elevated ($P<0.05$) in SFB-positive mice. These findings suggest that intestinal microbiota, including SFB that is obtained during rederivation, can alter mouse model phenotypes.

O-14: Identifying new antimalarial targets using ENU mutagenesis.

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The malaria parasite has a red cell lifecycle and it is during this part of its life that it causes significant harm to it host by causing severe anemia and cerebral malaria. Most current antimalarials target the erythrocytic stage of the parasite. This has led to the development of drug resistance by the parasite and this is universal; all antimalarials are experiencing resistance by the parasite to the level where only a small number of drugs are still useful.

We are developing a novel approach to the development of antimalarial drugs where we target the host and not the parasite. There is good evidence in nature that mutations in the red cell can confer resistance to a malarial

infection and that these are long-lived. We are therefore identifying mutations in mice that cause the mouse to survive an otherwise lethal dose of a murine malaria (*P. chabaudi*). This is a dominant screen and mice with mutations that cause survival are examined extensively to identify the mechanism of resistance. The mutation is identified through exome sequencing. We have around 30 mutant survival lines. We also screen mice for red cell abnormalities and have around 40 such lines. About half of these are resistant to death from a malarial infection.

We will describe several interesting mutants and make some observations about the nature of mutation that renders an animal resistant to malarial infection.

O-15: Identification of a novel ENU-induced mutation affecting plasma triglyceride levels in mice

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Using whole-exome and RNAseq analyses, we have identified an ENU-induced mutation associated with elevated plasma triglyceride levels. This novel, autosomal recessive mutation (*hlb444*), arising on a C57BL/6J background, is a T to A missense mutation in a FAST kinase domain containing protein, *Fastkd5*. The change introduces a premature stop codon at amino acid 14 of 807 in the full-length protein. Linkage analysis reveals the mutation displays almost complete penetrance.

FASTKD5 is a highly conserved member of the FAST protein family shown to localize to mitochondria in humans, but whose function remains largely unknown. Results from studies using other FAST family members suggest that these proteins are involved in various aspects of mitochondrial respiration and may participate in the regulation of cellular energy balance. The role of FASTKD5 in lipid metabolism has yet to be elucidated. Additional studies are ongoing to examine the response of the mutant to a high fat diet, investigating FASTKD5 expression in other tissues, analysis of mitochondrial function and further elucidating this protein's role in mouse models of metabolic syndrome.

We presume this protein is involved in cellular respiration and suspect that it is either critical to lipid catabolism, or is involved in mitochondrial fatty acid transport. Additional data from ongoing experiments will be presented as well as additional insight to an as yet undescribed role for this protein in the regulation of plasma lipids.

O-16: Positional cloning of the Chr 15 Quantitative Trait Locus *Fob3b2* affecting leanness in mice

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Although today's »obesogenic« environment is characterized by nutrient excess coupled with physical inactivity, a relatively large proportion of the human population remains lean suggesting genetic resistance to obesity development. Our positional cloning study aimed at identifying a causal gene for the *Fob3b2* QTL that confers anti-obesity effects in a polygenic mouse model. We first fine mapped the genetic interval of *Fob3b2* QTL using F2 crosses of congenic lines, interval-specific haplotyping, comparative mapping and confined the causal leanness effect region to a small Lean-line segment (~2 Mbp) on mouse Chr 15. The segregated F2 population was treated with the high fat diet (HFD) to examine genotype-diet interactions. Statistically significant differences ($p < 0.0001$) in body mass and various fatness traits were obtained between the FF (homozygotes for "Fat" alleles) and LL (homozygotes for "Lean" alleles) F2 genotypes. Significant and more pronounced effects were obtained in females suggesting a gene-sex interaction. Furthermore, F2 animals homozygous for the Lean-line segment exhibited improved glucose tolerance and insulin sensitivity. Gene expression and functional analyses of the ~20 positional lean gene candidates identified the nuclear-encoded mitochondrial thiosulfate sulfur-transferase (*Tst*, rhodanese) as the only upregulated adipose-specific gene mapping to the *Fob3b2* interval. Sequencing of transcribed regions revealed an SNP in the 3'UTR potentially affecting a miRNA binding site. Allelic imbalance test was performed on F2 heterozygotes in the *Fob3b2* segments, and showed significantly higher (1.64-fold) expression of the Lean-line *Tst* allele in the adipose tissue. Our genetic, transgenic and functional analyses as well as collaborative human genetics results strongly suggest that we identified a novel gain-of function adipose-derived lean gene potentially applicable for treatment of obesity and related metabolic disorders.

O-17: Fine-mapping a trans-eQTL hotspot

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Efforts to map the genetic loci (called eQTL) that influence gene expression have identified local-eQTL, near the genomic location of the influenced gene, and trans-eQTL, far away from the influenced gene. In many cases, trans-eQTL "hot spots" have been identified: a genomic region that influences the expression of many genes, genome-wide.

We have been investigating an intercross between the diabetes-susceptible mouse strain BTBR and the diabetes-resistant strain C57BL/6J (B6). The ~500 offspring mice were all leptin knockouts, and have genome-wide gene expression data on six tissues. We identified numerous trans-eQTL hotspots, many of them tissue specific. Of particular interest is a well-defined hotspot on Chromosome 6, which influenced the expression of >2500 genes in pancreatic islets.

In order to define the hotspot interval, we used mice that were not recombinant in the region of the eQTL to build a classifier for predicting eQTL genotype from the pattern of expression of genes mapping to the region. We then applied this classifier to infer the eQTL genotype of mice that were recombinant in the region of the eQTL. By comparing the predicted eQTL genotypes with observed genotypes at the markers in the region, we defined the eQTL interval as 141.5 - 144.9 Mbp [length = 3.4 Mbp], containing 23 protein-coding genes.

To fine-map the locus, we genotyped an additional 5 markers on 28 mice with recombination events in the eQTL interval. This narrowed the interval to 141.7 - 142.3 Mbp [length = 0.6 Mbp]. This interval contains just seven genes, including islet amyloid polypeptide (*Iapp*), which has been suggested to induce apoptotic cell-death in particular cultured cells, an effect that may be relevant to the development of type II diabetes.

O-18: Combined sequence-based and genetic mapping analysis of complex traits in outbred rats

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The rat is an important model for many human diseases, particularly where its physiology more closely resembles that of humans than does the mouse. We carried out a combined sequence and genetic mapping analysis of 160 complex phenotypes including a number of models of diseases, in 1400 outbred (Heterogeneous Stock) rats [ref1].

The rat Heterogeneous Stock is descended from 8 inbred strains through more than 60 generations of circular breeding during which recombination events have accumulated. As a result, the genetic factors contributing to phenotypic variation can be precisely mapped to the genome. We mapped 355 quantitative trait loci (QTLs) for 122 phenotypes, with 90% confidence intervals on average 4.5Mb wide.

We fully sequenced the eight founder strains and used that information to identify the genetic variants and genes underlying these QTLs. We identified 35 causal genes involved in 31 phenotypes, implicating new genes in models of anxiety, heart disease and multiple sclerosis. The relationship between sequence and genetic variation is unexpectedly complex: at approximately 40% of QTLs, a single sequence variant cannot account for the phenotypic effect.

Using comparable sequence and mapping data from mice, we show that the extent and spatial pattern of variation in inbred rats differ substantially from those of inbred mice and that the genetic variants in orthologous genes rarely contribute to the same phenotype in both species.

1. Baud, A. *et al.* Combined sequence-based and genetic mapping analysis of complex traits in outbred rats. *Nature Genetics* **45**, 767-75 (2013).

O-19: Caudal regression in Danforth's short tail is caused by the ectopic expression of *Ptf1a* induced by the insertion of a transposon.

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Human caudal regression syndrome (CRS) is a rare congenital disorder in which lumbosacral anomalies are combined with anorectal and urogenital malformations. Danforth's short tail (*Sd*) is a semidominant mutation on mouse Chromosome 2, characterized by spinal defects, urogenital defects and anorectal malformations and is a model for CRS. In this study, we identified the genetic bases for *Sd* mutation involved in the pathogenesis of *Sd*

phenotype. We used a positional cloning approach to identify *Sd*. Initially, to create a cosmid contig of the equivalent *Sd* region, a genomic cosmid library generated from homozygous *Sd* embryos was screened. Interestingly, we found one cosmid clone whose insert size was bigger than the putative size. We performed shotgun sequencing and reconstructed from the reads using sequence assembly software using the candidate cosmid clone. Surprisingly, the genomic DNA in this cosmid clone contained an early transposon (ETn) near the *Gm13344*, *Gm13336* and *Ptf1a* genes. To confirm whether the ETn insertion found in the *Sd* locus is responsible for *Sd*, we produced an ETn knock-in allele (kiETn) in WT ES cells. The established kiETn homozygotes showed characteristic *Sd* phenotypess. To examine the effects on transcription of *Gm13344*, *Gm13336* and *Ptf1a* gene, we performed RT-PCR. Unexpectedly, we found a fusion transcript containing the 1st and 2nd exons of *Gm13336* and the ETn sequence in *Sd* mutant embryos (mGm13336). Interestingly, RT-PCR analyses revealed increased expression of all four transcripts in the *Sd* mutant. To determine whether any of these four transcripts is responsible for *Sd*, we generated lines of transgenic mouse carrying either a proximal genomic fragment or a distal genomic fragment. The transgenic mice with the ETn- *Gm13336/Ptf1a* showed a short tail similar to the *Sd* mutant. Our current research revealed that an insertion of ETn caused *Sd* and *Gm13336/Ptf1a* region was responsible for *Sd*.

O-20: A Visualization Tool For Exploring the Gene Expression Landscape in a Full Three-Founder Diallele

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In a recent experiment, we performed a gene expression analysis in both sexes of a full three-way diallele cross of the wild-derived laboratory mouse strains: WSB/EiJ, PWK/PhJ, and CAST/EiJ. A total of 90 samples were analyzed via RNA-sequencing on brain tissue and microarrays for brain, liver, lung, and kidney. For RNA-seq, the data is presented as normalized Total Read Count (TReC) and Allele-Specific Read Count (ASReC). For microarray data, the data is presented as normalized intensity values. Within this dataset, we observed many interesting examples of parent-of-origin, strain effects, and cis effects. To present these findings, we created the Gene Expression in the Collaborative CrOss (GECCO) web tool for exploring the experiment data.

GECCO provides access to the data through both browsing the mouse genome and by specific gene lookup. Both techniques lead to a gene expression viewer that shows the expression levels (TReC or intensity) of each sample in the experiment. The samples are clustered such all the samples of the same type are grouped, and then a box-and-whisker plot is overlaid on the data. These are further grouped such that founders are separate and F1 crosses are beside their reciprocal cross. For RNA-seq data, there is an additional view that shows the ratios of ASReC for each F1 sample in the experiment.

These views allow for quick observation of many known gene effects. Parent-of-origin effects are viewable by comparing a cross to its adjacent reciprocal cross and/or by looking at the ASReC ratios for the two crosses. Overdominance and underdominance can also be visually observed by comparing the expression of the founders to the F1 crosses. The genome browser can also be useful for identifying regions where the genes all have similar expression levels. The GECCO tool is currently publicly accessible through http://csbio.unc.edu/gecco/.

O-21: A Large Scale Reverse Genetic Screen to Identify Novel Skin Phenotypes in Mouse Mutants

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Permanent stop-and-shop mouse mutant resources provide an excellent platform to decipher phenogenomics of major tissues in the mice. With the availability of large number of mouse mutants, it is now possible to study the function of mammalian genes at unprecedented high number. Here we present utilization of such resources to screen phenotypes in the murine skin. We have analysed tissue from more than 500 knockout mouse mutants that are generated from Sanger Mouse Genetics Project as part of IKMC (International Knockout Mouse Consortium) at the Wellcome Trust Sanger Institute, UK. Tail skin of these knockout mice were analysed systematically to screen for phenotypes, which ranged from subtle to strong. This screen enabled us to identify known and unknown genes involved in mammalian skin homeostasis. We have mapped several human skin disorders with available mutant mice based on their skin phenotype. We have also clustered the mutants into major signalling pathways to reveal their role. The resources generated from this study further be utilized to elucidate dermal and epidermal cross talks and tissue microarray based functional screenings. These resources are intended for the access to the wider scientific community. To our knowledge, a pilot scale collaborative initiative that focused on a tissue level phenotype screen has not been reported so far. In this presentation, I will discuss screening strategy, methods, findings, extended analysis of three skin phenotypes and future direction of this collaborative project.

O-22: Pervasive Allelic Imbalance Revealed By Allele-Specific Gene Expression In Highly Divergent Mouse Crosses

James J Crowley*[1], Vasyl Zhabotynsky[2], Wei Sun[2], Shunping Huang[3], Isa Kemal Pakatci[3], Yunjung Kim[1], Jeremy R Wang[3], Andrew P Morgan[1], John D Calaway[1], David L Aylor[1], Zaining Yun[1], Timothy A Bell[1], Ryan Buus[1], Mark E Calaway[1], John P Didion[1], Terry J Gooch[1], Stephanie Hansen[1], Nashiya N Robinson[1], Ginger D Shaw[1], Jason Spence[1,8], Corey Quackenbush[1], Cordelia Barrick[1], Yuying Xie[1], William Valdar[1,4], Alan B Lenarcic[1,4], Wei Wang[3,9], Catherine E Welsh[3], Chen–Ping Fu[3], Zhaojun Zhang[3], James Holt[3], Zhishan Guo[3], David Threadgill[7], Lisa M Tarantino[5], Darla R Miller[1], Fei Zou[2], Leonard McMillan[3], Patrick Sullivan[5,6], and Fernando Pardo–Manuel de Villena[1,4,6]

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The widespread localization of disease-associated variation within regulatory DNA underscores the importance of cis regulatory variation in humans. A thorough annotation of cis regulatory variants in the laboratory mouse in diverse tissues is needed to understand the function of the human genome. Here we provide such a detailed portrait using RNA sequencing in all possible combinations of a three-way diallel. Tissue was the greatest predictor of gene expression, followed by strain, parent-of-origin and sex. In brain, we observed cis regulatory effects in 11,633 genes (88% of testable genes), exceeding all mouse eQTL studies to date. Cis-regulated brain-expressed genes were enriched for behavioral and neurological phenotypes in knockout mice as well as peripheral blood and brain eQTLs for orthologous human genes. We estimate that at least 1 in every 1,000 SNPs creates a cis eQTL, similar to the fraction of SNPs predicted to alter protein function. For parent-of-origin effects, we identified 98 imprinted genes in brain. We observed that imprinting is incomplete for most genes, and that cis acting mutations can modify the strength of imprint. We confirmed dosage compensation equalizing expression between males and females and between Chromosome X and autosomes. We conclude that pervasive regulatory variation underlies complex genetic traits in mice. Our results, accessible as an online catalog (http://csbio.unc.edu/gecco), underscore the importance of laboratory mice to compliment human GWAS.

O-23: RNA-seq alignment to individualized diploid transcriptomes reveals extensive local genetic regulation and differential allelic expression in outbred DO mice.

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Genetic variation that deviates from a reference genome sequence can create biases in the alignment of short sequencing reads and can distort transcript abundance estimates in RNA sequencing (RNA-seq) experiments. Mapping bias is amplified in highly diverse populations such as the Collaborative Cross (CC) recombinant inbred strains and Diversity Outbred (DO) outbred population, and error tolerant alignment alone cannot correct this problem.

We hypothesized that alignment of sequence reads to the imputed transcriptomes of individuals (individualized transcriptomes) from which tissue samples were obtained would reduce alignment errors and improve transcript quantification. We developed a method, implemented as the software package Seqnature, that constructs individualized genomes and transcriptomes of experimental model organisms including inbred mouse strains and genetically unique outbred animals. We first align each short read to a pool of all possible isoform sequences in that sample (with individual-specific SNPs and indels included), and then estimate transcript abundance with an EM-based method. For diploid samples, two versions of each isoform sequence are included in the search space, and we can directly estimate gene and isoform abundance for each allele.

We show that alignment of liver RNA-seq reads from a large population of DO mice to individualized diploid transcriptomes 1) increases read mapping accuracy, 2) improves transcript abundance estimates, and 3) corrects erroneous trans linkages and unmasks thousands of hidden cis associations in an application to expression QTL mapping. We observe differential allelic expression in a majority of all expressed genes, providing evidence that transcription is highly sensitive to local genetic variation. The Seqnature software and associated analytical pipeline may be applied to mice (including inbred strains, F1 hybrids, F2 intercross, and outbred stocks), other model organisms (e.g. fly, worm), and human RNA-seq data.

O-24: The Architecture of Parent of Origin Effects in Mice

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The scope of parent of origin effects on complex traits is largely unquantified. Here we show that in outbred mice, most phenotypes involve genome wide parent of origin effects. This observation apparently contradicts predictions that the number of imprinted genes is small. We investigate two non-imprinted candidate genes, *Man1a2* and *H2-ab1,* at imprinted QTL using reciprocal F1 crosses of knockouts, to understand how parent of origin effects arising outside of imprinting control regions, influence phenotype and gene expression. We show that the expression of many genes is altered in a sex-, tissue- and parent of origin- specific manner, affecting genomic clusters of genes, and crucially known imprinted genes. We identify 21 new genes with parent of origin related hippocampal expression. We deduce that non-imprinted genes generate parent of origin effects by interaction with imprinted loci, and that the importance of the number of imprinted genes is secondary to their network of interactions.

O-25: Compatibility of *Prdm9* alleles in the (PWD x B6)F1 Sterile Hybrids

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Incorrect epistatic interactions reduce fitness of intersubspecific F1 hybrids, because the diverged alleles of the interactors did not pass together via natural selection. However, the role of interallelic interactions in animal hybrid fitness is unclear. The PR-domain 9 (PRDM9) is a histone 3 methyltransferase that determines the sites of meiotic cross-overs necessary for successful meiosis. The *Prdm9* gene participates in meiotic arrest of spermatogenesis in the offspring of PWD females and B6 males (*Mus m. musculus* x *M. m. domesticus*)F1. These hybrid males carry no sperm. Removal as well as overexpression of the $Prdm9^{B6}$ allele significantly increases the fertility of these F1 males. The (PWD x B6)F1 model of hybrid sterility requires PWD/B6 heterozygosity at proximal Chromosome 17, the location of *Prdm9*, suggesting that its interallelic incompatibility could participate in these phenotypes. To validate this hypothesis, we transferred a $Prdm9^{tm1Ymat}$ knock-out to PWD background and prepared intersubspecific F1 males carrying the null $Prdm9^{PWD}$ allele. The $Prdm9^{PWD}$ deletion did not rescue fertility of azoospermic (PWD x B6)F1. We also verified our results by phenotyping the F1 male offspring of a female carrying $Prdm9^{B6}$ on PWD background and a B6 male harboring another *Prdm9* null allele, $Prdm9^{tm1Fore}$. These (PWD x B6)F1-$Prdm9^{B6}$/$Prdm9^{tm1Fore}$ males were also azoospermic, indicating that the $Prdm9^{PWD}$ allele and the $Prdm9^{PWD}$-$Prdm9^{B6}$ interallelic interactions play a minor role in our model of hybrid sterility compared to the effect of $Prdm9^{B6}$.

O-26: A novel meiotic drive system in the mouse gives rise to a selective sweep in the absence of changes in fitness

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Statistically significant departures from expected Mendelian inheritance ratios (transmission ratio distortion, TRD) have been observed in experimental crosses and natural populations of plants and animals, including mice and humans. TRD can have profound consequences on population allele frequencies, including fixation of the favored allele (selective sweep). We and others have previously reported TRD of Chr 2 in favor of a wild-derived allele (WSB/EiJ) in the Collaborative Cross (CC) mouse panel. Our data show that TRD in the Diversity Outcross (DO) population is three-fold greater than in the CC. We mapped the region of maximum TRD (responder to drive on Chr 2, *R2d2*) to an interval ~45 cM from the centromere (78-86 Mb); we are fine-mapping the causal locus by crossing individuals that are recombinant within this interval. The observed TRD may be caused by differential viability of gametes, embryos or individuals of either sex, or unequal segregation of chromosomes during female meiosis (meiotic drive). In multiple crosses segregating for *R2d2*, we observe TRD when the WSB allele is transmitted through the maternal germline. In contrast, there is no evidence of transmission bias in sires. Furthermore, we do not observe any correlation between TRD and survival, fertility or fecundity. These results strongly support that TRD of the $R2d2^{WSB}$ allele is caused by a novel female meiotic drive system in the mouse. Additionally, the level of maternal TRD varies depending on the genetic background. This suggests an epistatic

interaction between *R2d2* and at least one modifier locus. We have observed transmission frequencies of $R2d2^{WSB}$ up to 95%, making this this strongest mammalian meiotic drive system that has been described. The $R2d2^{WSB}$ allele has rapidly swept through the DO population and without intervention would become fixed within another 18 generations. Remarkably, this hard selective sweep is occurring in the absence of any changes in organismal fitness.

O-27: Mouse *Slc9a8* mutants exhibit retinal degeneration due to the misregulation of endosomal pH

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The Sanger Institute Mouse Genetics Project has screened over 640 mouse lines homozygous or heterozygous for a knockout first allele derived from ES cell targeting. The screen includes both slit lamp examination of the anterior segment of the eye and ophthalmoscope examination of the posterior segment followed by retinal imaging. Mice homozygous for the $Slc9a8^{tm1a(KOMP)Wtsi}$ allele were identified with abnormal retinal pigmentation consistent with retinal degeneration. The screen found no other abnormal phenotype from >280 diverse characteristics assessed. *Slc9a8* encodes the solute carrier family 9, (sodium/hydrogen exchanger), member 8 also called NHE8. Previous work focused on the role of this protein in the kidney and intestine and little is known about the role of NHEs in the eye.

Sodium hydrogen exchange is required for the eye to maintain intraocular pressure. However, we measured the IOP in mutant mice and find it no different from wild type littermates. Histological examination of mutant eyes showed some photoreceptor degeneration from 8 weeks of age, although even at 12 months some photoreceptors remained. Electroretinography reveals a significant reduction in response to light, suggesting a functional defect in the photoreceptors in addition to cell loss. Both histology and scanning laser ophthalmoscopy indicate a defect in the retinal pigment epithelium. We made an RPE-specific knockout, which recapitulated the retinal degeneration of the constitutive knockout, indicating that loss of SLC9A8 in the RPE is sufficient to promote photoreceptor loss.

SLC9A8 belongs to the organellar class of exchange proteins which mediate ion exchange, maintain luminal pH and are involved in intracellular membrane trafficking. In agreement with earlier work we find it localised to the trans-golgi network and endosomes, which in mutant cells are abnormal in size and pH. Defective endosome recycling in the RPE may lead to cell death or dysfunction, which in turn will affect the photoreceptors.

O-28: SEC23B deficient humans and mice exhibit different phenotypes

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Congenital dyserythropoietic anemia type-II (CDAII) is an autosomal recessive disorder of erythroid maturation resulting from *SEC23B* mutations. SEC23A/B are paralogous components of COPII vesicles, which transport proteins from the Endoplasmic Reticulum to the Golgi apparatus. Mice homozygous for a *Sec23b* genetrap-allele (C57BL/6J-$Sec23b^{Gt(AD0407)Wtsi}/Sec23b^{Gt(AD0407)Wtsi}$) die perinatally with pancreatic degeneration. To examine the impact of SEC23B-deficiency on adult murine hematopoiesis, $Sec23b^{Gt(AD0407)Wtsi}/Sec23b^{Gt(AD0407)Wtsi}$ or wildtype fetal liver cells (FLC) from E17.5 embryos were transplanted into lethally-irradiated C57BL/6J mice. Recipients of C57BL/6J-$Sec23b^{Gt(AD0407)Wtsi}/Sec23b^{Gt(AD0407)Wtsi}$ FLC exhibited no CDAII and were indistinguishable from wildtype transplant controls. To test for a subtle hematopoietic defect, $Sec23b^{Gt(AD0407)Wtsi}/Sec23b^{Gt(AD0407)Wtsi}$ and wildtype GFP+ FLC were co-transplanted (1:1 ratio) into lethally-irradiated recipients, with no competitive difference observed. Two independent *Sec23b* bacterial-artificial-chromosome transgenes demonstrated complete rescue of the $Sec23b^{Gt(AD0407)Wtsi}/Sec23b^{Gt(AD0407)Wtsi}$ phenotype, ruling out a passenger gene mutation. A *Sec23b* conditional-allele ($Sec23b^{tm1c(EUCOMM)Wtsi}$) was also generated. Mice with erythroid-specific SEC23B-deficiency (generated using C-$Epor^{tm1(EGFP/cre)Uk}$ mice), exhibit no anemia. Transplant recipients of FLCs from E16.5 germline null C57BL/6J-$Sec23b^{tm1c(EUCOMM)Wtsi}/Sec23b^{tm1c(EUCOMM)Wtsi}$ embryos also exhibited no anemia. Pancreas-specific *Sec23b*-knockout mice (generated using STOCK $Ptf1a^{tm1.1(cre)Cvw}$/Mmnc mice or B6.FVB-Tg(Ipfl-cre)6Tuv/JPdx1 transgenic mice) exhibit a phenotype indistinguishable from complete SEC23B-deficiency, demonstrating that loss of pancreatic *Sec23b* expression is sufficient to explain the perinatal-lethality of C57BL/6J-$Sec23b^{Gt(AD0407)Wtsi}/Sec23b^{Gt(AD0407)Wtsi}$ and C57BL/6J-$Sec23b^{tm1c(EUCOMM)Wtsi}/Sec23b^{tm1c(EUCOMM)Wtsi}$ 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%), this suggests that the tissue-specific functions of SEC23A and SEC23B have shifted during evolution between humans and mice, and that CDAII may result from murine SEC23A-deficiency or combined SEC23A/B-deficiency. We are currently characterizing SEC23A-deficient mice. Further studies of the overlapping functions of SEC23A/B and their protein cargos should provide new insights into CDAII pathogenesis.

O-29: In mice with activated AKT, constitutive reduction of MTOR reduces the expression of *Cdk6* and delays the development of thymic lymphomas

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The PI3K/AKT/MTOR signaling pathway is hyperactivated in a variety of solid tumors and hematologic malignancies. This hyperactivation often results from overexpression or mutation/loss in PI3K, AKT, or PTEN. MTOR is a central downstream target of the pathway and is a critical modulator of cell cycle, size, proliferation, and metabolism. To examine the role of MTOR in the development of tumors induced by activated AKT, Tg(Lck-Akt2*)1Test (Lck-MyrAkt2) mice, whose constitutively active AKT induces thymic lymphomas, were crossed with *Mtor*$^{tm1.1Lgm}$ mice, which constitutively express reduced levels of MTOR protein. As expected, Lck-MyrAkt2 mice with wild type *Mtor* expression spontaneously developed thymic lymphomas at 10 – 20 weeks of age (with an average age of 14.5 weeks for palpable tumors). In the Lck-MyrAkt2 mice with reduced *Mtor* expression, tumor development was delayed (with an average age of 26 weeks for palpable tumors), and the mice lived longer, with a median survival of 181 days, which was three months longer than the mice with normal *Mtor* expression. The frequency of t14;15 translocations was similar in both groups of lymphomas. We performed gene expression profiling (GEP) of tumors arising from both groups of mice to uncover underlying mechanisms of reduced tumorigenicity seen in mice with low *Mtor* expression. Molecular and functional enrichment analysis of the genes found to be differentially expressed between the two groups identified cell death and survival and DNA replication and repair as the most enriched networks. *Cdk6* expression was 10-fold lower in the tumors from the mice with low *Mtor*. *Cdk6* and *Akt* are frequently abnormally expressed in human T cell lymphoblastic lymphomas and may contribute to the pathogenesis of this malignancy. We conclude that reduced levels of *Mtor* can inhibit tumor formation by activated AKT, and that CDK6 may be a critical target of this inhibition.

O-30: A suppressor screen in mouse *Mecp2* identifies cholesterol homeostasis as a therapeutic target in Rett Syndrome

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Mutations in methyl CpG binding protein 2 (MECP2) cause Rett Syndrome (RTT), the most severe autism spectrum disorder. *Mecp2* null mouse models recapitulate many of the symptoms of RTT and their study has provided insight into the physiological basis of disease. Reactivating silent *Mecp2* in symptomatic adult null mice reverses symptoms, suggesting that therapeutic intervention may be possible in RTT patients. Unfortunately, as a widespread epigenetic factor, MECP2 is extremely dosage sensitive, making direct manipulation a poor treatment option. MECP2 mutation impacts many biological pathways, but it is unclear which are relevant to symptom onset and progression. We used an unbiased forward genetic approach to identify dominant suppressors of phenotypes in *Mecp2* null male mice, dispensing with a priori beliefs about MECP2 function. Five suppressor mutations, which ameliorate symptoms of *Mecp2* loss, were identified using linkage mapping and whole exome sequencing. One is a loss-of-function mutation in squalene epoxidase (*Sqle*), a rate-limiting enzyme in committed cholesterol biosynthesis. The underlying basis for the rescue is a previously undescribed metabolic syndrome that develops in *Mecp2* mutant mice. Although lipid metabolism has not been implicated in the pathogenesis of Rett Syndrome, it is linked to many other neurological disorders. Further, MECP2 is required to link a repressor complex that regulates lipid homeostasis to DNA. Accordingly, *Mecp2* mutant mice were treated with cholesterol-lowering statin drugs, which alleviate motor symptoms and confer increased longevity in both males and females. Cholesterol metabolism therefore represents a potential new therapeutic target for the treatment of RTT. Our overarching goal is to identify a large number of suppressors through additional screening such that a list of potential therapeutic targets can be developed for RTT. A similar systems biology approach could be exploited to identify targetable pathways involved in other “untreatable” diseases, opening a new field for translational discovery.

O-31: Potent Modifiers of Intestinal Polyposis in Closely Related Inbred Mouse Strains

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The adenomatous polyposis coli (*APC*) tumor suppressor gene is mutated in Familial Adenomatous Polyposis (FAP), an inherited disorder that predisposes individuals to intestinal polyps and eventually leads to cancer. In the *Min* mouse model, the genetic background of mice carrying a mutation in the murine homolog of the *APC* gene (Apc^{Min}) is essential to the development of tumors, as inbred strains vary in their susceptibility to polyposis. Although complex trait analyses have identified several loci that modify intestinal tumor number and size in Apc^{Min}/+ mice, less than a handful of modifier genes have been identified to date. Unlike traditional quantitative trait loci (QTL) studies that exploit the diversity among evolutionarily distant inbred strains, we took advantage of genetic similarities between closely related inbred strains. Here we report the identification of protective modifier loci in the C57L/J (L) strain, which is closely related to the C57BL/6J (B6) strain. The F1 Apc^{Min}/+ offspring from intercrosses between L and B6 mice exhibited significant differences in polyp phenotypes compared to their B6 parents. Our QTL study revealed several autosomal regions that contain modifier genes. Candidate genes in two of these loci were investigated and several polymorphisms were prioritized for further study. We discuss the usefulness of this closely related strain approach in optimizing complex trait screens and speeding the process of causative gene identification. Research supported in part by NCI grants to LDS.

O-32: *CDCA7L* is a male-specific susceptibility locus and oncogene in glioma

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Astrocytoma and glioblastoma-multiforme (GBM) are the most common primary brain tumors and are currently incurable. Both astrocytoma and GBM show male-predominance, with a male-to-female ratio of 1:3. Previously, our lab has demonstrated that astrocytoma/GBM-tumorigenesis in the $Nf1^{tm1Tyj}$ $Trp53^{tm1Tyj}$/+ (*NPcis*) mouse model shows gender-bias in certain genetic contexts. Using linkage-analysis in the *NPcis* mouse, we have identified a male-specific genetic modifier locus on distal Chromosome 12, named *Arlm1* for astrocytoma resistance locus in males 1. To identify candidate genes for the *Arlm1* modifier of glioma risk, we used combinational bioinformatics approaches and cross-species comparisons, identifying cell division cycle associated 7 like (*Cdca7l/CDCA7L*) as a strong candidate for a male-specific astrocytoma susceptibility gene among 503 candidate genes. To investigate the role of *CDCA7L* in astrocytoma/GBM, we analyzed *CDCA7L* in both human GBM and mouse astrocytoma cell lines in both genders. *CDCA7L* expression was upregulated in GBM and astrocytoma cells compared to normal brain with male-predominance. shRNA-mediated *Cdca7l/CDCA7L*-silencing in male-derived mouse astrocytoma cells and human GBM led to decrease of both cell growth and viability. Further mechanistic study revealed that *Cdca7l/CDCA7L*-depletion in male-derived mouse astrocytoma cells and human GBM cells was connected with the induction of caspase 3-activation and reduction of cyclin D1-expression, suggesting the oncogenic role of *CDCA7L* in astrocytoma/GBM. On the other hand, *Cdca7l*-overexpression in female-derived mouse astrocytoma cells showed less degree of cell proliferation and viability increases. Strikingly, *CDCA7L*-depletion in human female-derived GBM cell line, U87MG caused increase of both cell growth and viability. These data highlight the sex-specificity of *CDCA7L* in astrocytoma/GBM tumorigenesis and provide evidence for the first modifier of male-specific susceptibility to brain tumors.

O-33: Rapid conversion of EUCOMM / KOMP-CSD alleles in mouse embryos using a cell-permeable cre recombinase

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The goal of the International Mouse Phenotyping Consortium (IMPC) is to generate knockout strains for all protein-coding genes in the mouse on a C57BL/6N genetic background, and to elucidate gene function by use of a broad spectrum high-throughput primary phenotyping screen. These phenotypes can then be studied in more depth by the scientific community at large with specialized areas of interest. To this end, the IMPC makes use of the EUCOMM/KOMP-CSD embryonic knockout stem cell collection.

We describe the use of a cell-permeable cre to efficiently convert the EUCOMM / KOMP-CSD knockout-first conditional-ready *tm1a* allele to the non-conditional lacZ-tagged null *tm1b* form in early mouse embryos in a high-throughput manner, consistent with the requirements of the IMPC-affiliated NIH KOMP2 project. This method results in rapid allele conversion and minimizes the use of experimental animals when compared to our CMV-cre based transgenic mouse breeding, resulting in a significant reduction in costs and time with increased welfare benefits.

O-34: Targeted chromosomal inactivation of the mouse *Tyr* insulators with engineered nucleases

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Chromatin boundaries, or insulators, are regulatory elements capable of dividing expression units into independent topological domains, allowing its function in the proper tissue and in the proper developmental stage, despite the epigenetic marks of the chromatin nearby. We identified two of such elements in the mouse genome, in the intergenic space in between of three differently-expressed genes: *Nox4*, *Tyr* and *Grm5*. When tested in plasmid-based assays, aimed to quantitate the insulator function in an artificial system, these sequences showed values comparable to the most active known insulator, the chicken HS4 insulator.

Nevertheless, alike other regulatory elements such as enhancers, insulators may fail to function when used ectopically. For example, in mouse transgenesis the efficacy of an insulator can vary dramatically according to the promoter used to drive the transgene. This variability reflects the interdependence between the insulator and the genomic context, the latter being the driving force for the first to appear and be conserved in the genome.

With this in mind, we decided to study the mouse *Tyr* insulators in their full genomic context. First, with Chromosome Conformation Capture (3C) we described the topological domain imposed by the *Tyr* insulators to the locus. Second, we designed engineered nucleases to produce the targeted inactivation of the *Tyr* insulators in transgenic mice.

Using the latest technology, namely TALEN and CRISPRs/Cas9 nucleases, we succeeded in deleting these sequences in cultured cells. We are currently applying our best performing nucleases for the generation of two transgenic mouse lines, in order to observe the expression patterns of *Nox4*, *Tyr* and *Grm5*, in different tissues and developmental stages, in the absence of the *Tyr* insulators.

O-35: Functional deciphering of genomic boundaries associated to cellular biological requirements

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In mammalian genomes, it is not uncommon to find a gene with a very restricted expression pattern right next to a ubiquitously-expressed one. This is possible thanks to the action of genomic boundaries or insulators, which delimit expression domains and prevent undesirable crosstalk between the regulatory elements of adjacent domains. Insulators can also be found partitioning the chromatin into active euchromatic and silenced heterochromatic regions, as well as flanking and protecting clusters of co-expressed genes. In order to find boundaries in a genome-wide fashion in the mouse genome, we explored these scenarios where the presence of insulator elements is necessary for the correct functioning of the cell. Several bioinformatic algorithms were developed and the resulting putative insulator sequences were functionally validated using various assays. Their enhancer-blocking properties were tested first in vitro in human cells in culture, and then in vivo in zebrafish. Additionally, we analyzed the ability of the most powerful element to protect from chromosomal position effects in transgenic mice. The description and characterization of new genomic boundaries would aid, not only in the understanding of how genomes are organized, but also in the improvement of the gene transfer technologies.

O-36: Examination of the effect of a DNA repair defect on the efficiency of ENU mutagenesis

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ENU mutagenesis is a powerful method for generating novel lines of mice that are informative with respect to both fundamental biological processes and human disease. Rapid developments in genomic technology have made the task of identifying causal mutations by positional cloning remarkably efficient. One limitation of this approach remains the mutation frequency achievable using standard treatment protocols, which currently generate approximately 1 sequence change per megabase when optimized. In order to increase this efficiency, we have initiated a study in which mice carrying a mutation in the DNA repair enzyme *Msh6* are treated with ENU. Of note, heterozygous mice tolerate treatment well, while homozygous mutant mice did not survive, even at lower doses. Mutation efficiency will be assessed by next-generation sequence analysis of the G1 progeny; exome sequencing will be done to sample the mutagenized genomes with high fidelity in an unbiased and cost-effective manner. As part of the same study, and using the same method for genome sampling, we will examine whether serial treatment of progeny of mutagenized mice can yield founders carrying large numbers of heterozygous mutations. We presently have multiple independent lines, both heterozygous for *Msh6* and wild-type, which are on the third round of ENU treatment.

O-37: Suppression and quantitative control of meiotic recombination hotspot activity

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Meiotic recombination is a fundamental biological process that ensures production of haploid gametes and increases genetic diversity. In mammals, recombination occurs at specific sites, known as hotspots, whose positions are determined by *Prdm9*. While the role of *Prdm9* in hotspot activation is well established, the mechanisms regulating other aspects of hotspot activity, including suppression and quantitative regulation, have remained unclear. Here we show that *Prdm9* regulates these phenomena as well. The recombination hotspot *Ush2a* is suppressed in F1 of CAST/EiJ x C57BL/6J mice and active only on a pure C57BL/6J background. To identify the suppressing factor, we measured *Ush2a* hotspotactivity in mice containing various combinations of *Prdm9* alleles, when the rest of the genetic background was either homozygous B6/B6 or heterozygous B6/CAST. In all crosses, the presence of the *Prdm9* CAST allele was necessary and sufficient to suppress *Ush2a* hotspot activity. To genetically map quantitative regulators, we followed the behavior of two hotspots whose activity is either increased (*Esrrg2* hotspot*)* or decreased (*Pbx1* hotspot) in the presence of distant CAST/EiJ alleles in a segregating F2 cross between C57BL/6J and CAST/EiJ. We quantitated hotspot activity using a new high-throughput sequencing method. A single QTL on Chr 17, overlapping with *Prdm9*, was responsible for the change in activity at the*Pbx1* hotspot. In addition, ChIP-Seq analysis of H3K4me3, the chromatin modification catalyzed by PRDM9, shows that the quantitative control at the *Essrg2* hotspot is likely due to two closely spaced hotspots activated by the B6 and CAST alleles of PRDM9. Taken together, these results suggest that *Prdm9* is the primary determinant of the quantitative differences at recombination hotspots, and that suppression is an extreme case of quantitative control.

O-38: *Prdm9* variability and its effect on genetic recombination in a Robertsonian house mouse natural population

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Understanding the mechanisms underlying speciation has been of special interest in biology. Although chromosomal rearrangements have been shown to play an important role in the process, more recently, different studies have suggested that *Prdm9* might be involved in the speciation process as its highly mutable sequence determines the position, number and strength of recombination events. In this context, the main aim of this study is to shed light in the impact, if any, of chromosomal rearrangements and *Prdm9* genetic variability in recombination. In order to do so, we have analyzed meiotic recombination jointly with *Prdm9* sequence variability across the Barcelona Robertsonian (Rb) polymorphism zone, a wild house mouse population with a wide range of diploid numbers (from 2n=22 to 2n=40). Our analysis revealed the presence of new *Prdm9* allelic variants in the Barcelona population that show differences in both sequence and number of zinc finger repeats compared to previous studies. Our results also revealed a significant decrease in the number of crossovers per cell in Rb specimens, especially so in specimens with low zinc finger repeats. Based on these observations, we postulate that the presence of chromosomal rearrangements and the reduction in the number of *Prdm9* zinc finger repeats would influence the overall recombination rates that characterize Rb specimens.

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O-39: PRDM9-dependent modification organizes hotspot chromatin structure

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Meiotic recombination is restricted to 1-2 kb regions termed hotspots. In mouse and human, recombination at hotspots is initiated when the zinc finger protein PRDM9 binds to DNA and locally activates chromatin. This subsequently leads to introduction of DNA double-stranded breaks which are repaired using a non-homologous chromatid as a template. Previous genome-wide analysis of hotspots in mouse has focused on determining the position of meiotic double-strand breaks and found that most breaks overlap with PRDM9- modified chromatin. We now add to this understanding by providing a detailed picture of hotspot chromatin organization, confirmation that PRDM9 physically binds the identified motifs, and insight into downstream consequences of PRDM9-dependent modification on meiotic recombination. We combine genome-wide analyses of PRDM9 modification with fine-scale analysis of hotspots using chromatin immunoprecipitation with H3K4me3 antibody followed by high-throughput sequencing. To facilitate the identification of PRDM9-dependent chromatin modifications, we have compared the H3K4me3 pattern of C57BL/6J, containing $Prdm9^{b}$ allele, with that of a B6 knockin mouse strain, containing the $Prdm9^{wm7}$ allele. We find that PRDM9-trimethylated nucleosomes are symmetrically organized around a central nucleosome-depleted region. The latter extends for about 165 bp, much longer than the typical 35 bp spacer region between nucleosomes, and contains the predicted PRDM9 binding motif. We demonstrate that PRDM9 expressed in *E. coli*, does indeed bind the hotspot DNA sequence *in vitro* within this spacer region at the predicted motif. Importantly, this chromatin organization is a product of PRDM9

presence, as it only occurs at hotspots when the corresponding *Prdm9* allele is present. We find that meiotic crossing over is constrained within the trimethylated nucleosomes, suggesting that the new chromatin environment generated by PRDM9 is required for Holliday junction migration. These findings show that PRDM9 significantly alters the hotspot chromatin environment, potentially creating a permissible landscape for downstream meiotic recombination machinery.

O-40: Defects in *Nek8* result in abnormal specification of developmental patterning, polycystic kidney disease, and impaired response to replication stress

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The kinase *Nek8* is emerging as a multifunctional protein with roles in disparate cellular functions. We discovered *Nek8* in an analysis of the juvenile cystic kidneys (*jck*) model of PKD; affected mice carry a missense mutation in the presumptive regulatory domain. Missense mutations in this domain of *Nek8* have also been identified in patients with the renal cystic disease nephronophthisis (NPHP9) and in the Lewis polycystic kidney rat.

We generated a *Nek8*-null allele($Nek8^{tm1Bei}$); homozygous mice die at birth and exhibit randomization of left-right asymmetry. Ciliogenesis is intact in *Nek8*-deficient embryos and cells, but nodal ciliary signaling is perturbed as embryonic left-sided marker genes are misexpressed. We generated $Nek8^{jck}/Nek8^{tm1Bei}$ compound heterozygotes; these mutants develop less severe cystic disease than *jck* homozygotes, suggesting that the *jck* missense allele encodes a gain-of-function protein. Notably, *Nek8-/-* and *Pkd2-/-* embryonic phenotypes are strikingly similar. PKD2 (PC2) is expressed properly in *Nek8*-deficient embryos and cells; however, similar to cells lacking PKD2, *Nek8*-depleted IMCD cells exhibit diminished flow-dependent calcium influx, which suggests *Nek8* mediates PKD2-dependent signaling.

More recently, *Nek8* has been identified as an effector of the ATR (ataxia telangiectasia and Rad3 related) replication stress response. Cells lacking *Nek8* form DNA double-strand breaks, and NEK8 protein physically interacts with ATR and travels with the replication fork. Notably, elevated DNA damage signaling is evident in *jck* homozygous kidneys at 3 weeks of age, well prior to the point that they exhibit extensive cystic disease.

Thus, in addition to the well-established specific localization of NEK8 to the inversin compartment of the ciliary axoneme, it is also present in the nucleus and binds to chromatin. Functionally, it plays a role in mediating embryonic nodal signaling, maintenance of renal tubular integrity, and DNA damage response. It will be of interest to assess whether these activities reflect multiple independent *Nek8* activities, or are mechanistically related.

O-41: The ENU induced mutation, germ cell depletion 2 *(gcd2)*, is a missense mutation in *Kif18a* that causes cell-type-specific mitotic defects.

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Using high-throughput sequencing, we've discovered that the ENU-induced, *germ cell depletion 2* (*gcd2*) mutation is a missense mutation in *Kif18a*. KIF18A is a microtubule motor protein that is uniquely capable of controlling the plus-end spindle microtubule dynamics that are essential for mitotic chromosome congression and alignment during metaphase. In HeLa cells, loss of KIF18A causes mis-alignment of metaphase chromosomes, elongated spindles and activation of the spindle assembly checkpoint. The *gcd2* mutation leads to a conservative amino acid change (R308K) in a highly conserved arginine within the motor domain of *Kif18a*. To test the impact of this conservative amino acid change, we expressed a GFP tagged version of the orthologous, human mutant protein in HeLa cells and using live cell imaging found the phenotype to be identical to that of functional null alleles when compared to that of similarly GFP tagged wild type KIF18A. Specifically, mutant KIF18A was unable to accumulate at the plus-end of kinetochore microtubules, which resulted in increased chromosome oscillations at metaphase and ultimately, mitotic arrest. Therefore, we conclude that the $Kif18a^{gcd2}$ allele is a functional null. To examine the impact of this mutation in primary cells, we derived embryonic fibroblasts from mutant and control embryos. Cell cycle and growth curve analyses revealed severe proliferation defects in the absence of cell cycle arrest. Moreover, mutant MEFs, fetal germ cells and spermatogonia accumulated in M phase and exhibited defects in chromosome alignment. While *Kif18a* is broadly expressed and has an essential function in mitosis, we have yet to discover a phenotype outside of the developing germ line. Therefore, *Kif18a* appears to be one of a growing number of genes with generalized roles in genome dynamics that are uniquely required for germ cell development.

O-42: Rat Resource and Research Center

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The Rat Resource and Research Center (RRRC) was established in 2001 with funding from the National Institutes of Health. The goals of the RRRC are to (1) provide the biomedical community with a repository for valuable rat strains, and (2) shift the burden for maintaining and distributing rat models from individual investigators to a centralized repository. Currently, the RRRC has approximately 350 rat lines received through active recruitment of valuable rat models and donations from investigators. Upon importation of rat strains/stocks into the RRRC, gametes and embryos are cryopreserved to ensure against future loss of the model. Quality control measures include extensive genetic validation and pathogen testing. Models in the RRRC are distributed as live animals, and cryopreserved gametes and embryos. Additionally, rat embryonic stem cell lines are available. Research efforts by the RRRC have advanced cryobiology and assisted reproductive technologies for the rat. Due to high success rates with intra-cytoplasmic sperm injection, the RRRC uses sperm cryopreservation as a cost effective method for banking large collections of single gene mutations and ensuring reliable recovery when models are requested. The RRRC has expertise in rat reproductive biology, colony management, health monitoring, genetic assay development/optimization, and the isolation of germline competent ES cell lines from transgenic rats; our staff and researchers are readily available for consultation. The RRRC has a number of fee-for-service capabilities such as a wide variety of genetic analyses, cytogenetic characterization including spectral karyotype analysis, strain rederivation, and spermatozoa cryopreservation. Our website (www.rrrc.us) allows user-friendly navigation and provides information about all strains, cell lines, model donation procedures, on-line ordering, lists of services, publications, and protocols. The RRRC is a valuable resource for rat users as it continues to expand the number of accessible rat models and services available to the global biomedical community.

O-43: Status of The Diversity Outbred Population

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The Diversity Outbred (DO) population is a heterogeneous stock derived from the same eight founder strains as the Collaborative Cross inbred strains. This talk will summarize the status of DO colony, which is in the 14th generation of outbreeding as of May 2013. Several large projects are underway using DO mice to investigate genetics of complex traits. New genotyping resources and analytical tools have been developed to support these studies. Accumulation of recombination events in the DO is tracking predicted rate. However there was a major distortion in the founder allele frequencies driven by a selective sweep of WSB/EiJ allele on Chromosome 2. Corrective action was taken to restore allelic balance in this region. We will present empirical results and simulations to support sample size and other study design decisions. The DO is thriving and ready for widespread adoption by this community.

O-44: The Mouse Genomes Project: From sequence variation to complete 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 of the project involved deep sequencing of the strains and creating comprehensive catalogs of molecular variation ranging from single base changes up to large structural differences of multiple kilobases. We are now in phase 2 of the project with the focus being the production of accurate genome sequences and gene annotation for each of the strains. Therefore, in 2012 we resequenced each of the strains to between 40-80x coverage on the HiSeq platform and have been finalising the sequencing of large fragment libraries of varying sizes, and generating long range optical maps for a subset of the wild-derived strains. 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. Perhaps, the most interesting aspect of having genome sequences for the strains is the proportion of sequence not found in the C57BL/6J reference genome. We estimate that there is up to 4.5Mbp of new sequence in the strains and are currently examining this for coding sequences. Finally, we find that there is varying representation of the known forms of repeat elements (SINE, LINE, and ERVs).

O-45: The Next-Gen Mouse and the Missense Mutation Library delivering new resources for understanding human disease

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The Australian Phenomics Facility, The Australian Phenomics Network, The Australian National University, Canberra, Australia

The Australian Phenomics Network (APN) and Australian Phenomic Facility (APF) have capitalised on expertise in genome-wide mutagenesis and advances in DNA sequencing efficiency to create a new library of missense and nonsense alleles in the mouse [http://databases.apf.edu.au/mutations/]. Treatment of mice with the chemical mutagen N-ethyl-N-nitrosourea (ENU) efficiently generates random single-base mutations in the germline DNA. ENU-induced mutations are subtle (hypomorphic, dominant negative, gain-of-function) and model human single nucleotide variants (SNVs), the most common kind of disease-causing mutation. ENU mutagenesis is the most

powerful forward genetic strategy in the mouse. However, the dramatic fall in the cost of DNA sequencing renders ENU mutagenesis an attractive reverse genetic strategy without the need for ES cells. Exome sequencing of each first generation offspring of an ENU-treated male C57BL/6 mouse identifies on average 25 protein-changing de novo mutations in a heterozygous state. By mid-2013 the library contained over 16,000 SNVs, including 650 nonsense mutations, 2,200 predicted to disrupt splicing, and 5,000 that are probably damaging as predicted by PolyPhen-2. Bioinformatic analysis of the SNVs against human Multiz alignments to UCSC liftOver mutations into human coordinates identified five SNVs mapped to human genes from ClinVar. Additionally, over 2,100 SNVs correlate with Orphanet rare human disease-associated genes. Importantly, many of these alleles are live and can be rapidly accessed for specific phenotyping based on known disease associations. Researchers who maintain a secure Gene Watch account are alerted when alleles are found in their specific genes of interest and can respond while the Next-Gen Mouse is live. The Missense Mutation Library continues to grow and is expected to offer alleles in over 50% of genes by the end of 2013. More information about these and other resources at [apf.anu.edu.au] and [www.australianphenomics.org.au]. Supported by Australian Federal and State Government research infrastructure funding and by the APN partners.

O-46: Informatics for the International Mouse Phenotyping Consortium

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The International Mouse Phenotyping Consortium (IMPC) is generating and characterizing a knockout mouse strain for almost every protein-coding gene in the mouse genome. Data from a standardized, broad-based phenotyping pipeline is being centralized, analysed and integrated by the MPI2 consortium in the IMPC-Data coordinating center. Dedicated 'data wranglers' are working with the production centers to ensure proper transfer and quality control of data occurs. An automated statistical analysis pipeline is identifying knockout strains whose phenotype parameters have values that lie outside the normal range. Annotation with biomedical ontologies is allowing biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research. Data integration with other resources will provide insights into mammalian gene function and human disease. Users can now search the database to find new phenotype data from IMPC strains characterized at international centers and find the production status of a knockout strain for any gene. The community is invited to explore and provide feedback as we build the first comprehensive mammalian functional genomic catalog at: www.mousephenotype.org

O-47: Quantitative Trait Locus Mapping in Diversity Outbred Mice

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The search for genes underlying complex phenotypes has been greatly aided by genetic mapping in the mouse. Traditionally, mapping has been carried out in two parent intercrosses with limited mapping resolution. Relatively few of these studies have led directly to the discovery of a gene that regulates the phenotype of interest. In order to improve mapping resolution, advanced intercrosses and multi-founder crosses have been developed. Diversity Outbred (DO) mice were developed to overcome these limitations by combining high genetic diversity and fine recombination block structure in order to increase the chances of mapping a phenotype to a small region. The DO mice are derived from the same set of eight founder strains as the Collaborative Cross and are maintained as an outbred population. The task of reconstructing DO genomes and mapping requires specialized methods and software. Here, we describe software uses a hidden Markov model to provide a probabilistic reconstruction of individual DO genomes from intensity based analysis of genotyping microarray data. Genotype probabilities are used to map phenotypes in a mixed model that adjusts for the kinship among DO mice. The model outputs additive effects for each founder allele that can be used to reduce the number of candidate genes under a mapping peak. We provide a complete analytical pipeline, implemented as a freely available R package, to go from phenotypes and genotypes to candidate gene list.

O-48: KOMPute: Computational prediction of gene function and phenotypes for KOMP2

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The Knockout Mouse Project 2 (KOMP2; http://commonfund.nih.gov/KOMP2/) is an international effort to systematically phenotype mice carrying targeted mutations generated by the International Knockout Mouse Consortium (IKMC) in over 17,000 mouse genes. Generating baseline phenotype data for these mouse "knockouts" is key to understanding the connection between genotype and phenotype as 65% of the genes targeted by the IKMC have no prior knowledge of phenotype and 50% have no experimentally based functional annotation according to the Mouse Genome Informatics (MGI) database.

We report here the results of computationally based predictions of phenotypes and functions of the genes targeted by the IKMC. Our algorithm is based on our previously developed methods for predicting functional relationships and phenotype annotations for mouse genes through integrating heterogeneous data into a functional network. For the study reported here, we integrated 898 genome-scale datasets and curated annotations from the Mouse Genome Informatics (MGI) database for IKMC genes for which there was a vector, ES cell, sperm, embryo and/or mouse resource available. The phenotype and function predictions are accessible to the research community via the KOMPute database (www.kompute.org). Predictions from KOMPute can be used to prioritize phenotyping efforts for both initial high throughput screens and low throughput secondary phenotype screens.

KOMPute allows users to view all genes predicted to be associated with a specific Mammalian Phenotype Ontology (MP) term or Gene Ontology Biological Process term. Alternatively, users can search by gene symbol to retrieve all of the predicted phenotypes and biological functions for that gene. As results from the KOMP2 phenotyping pipelines become available through the KOMP2 Data Coordination Center, we will compare our predictions to experimentally validated phenotyping results.

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Poster Abstracts

P-1: *Oune*, a Missense Mutation in *Tbx6*, Causes Congenital Vertebral Malformations in the Rat

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Congenital vertebral malformations are relatively common in humans and domestic animals. Although knockout mice uncovered the molecular mechanisms of embryonic somite segmentation, the animal models for human congenital vertebral malformations largely remain unknown. In a *N*-ethyl-*N*-nitrosourea mutagenesis screen in Kyoto, the *Oune*/Kyo mutant rat was identified because of short and kinked caudal vertebrae. We first analyzed skeletons of newborn *Oune* mutants by alizarin red-alcian blue staining. *Oune*/+ rats show loss of most cervical vertebrae in addition to the malformations of caudal vertebrae. Further, hemivertebrae and fused vertebral blocks in lumber and sacral vertebrae were detected in heterozygous rats. In *Oune/Oune* animals, severe displacement of whole vertebrae leads to early embryonic lethal. These phenotypes suggested that *Oune* is a semidominat mutation. To identify the *Oune* causative gene, genetic mapping and positional candidate approach were performed. By using 202 N2 animals and 50 genome-wide microsatellite markers, the *Oune* locus was mapped to rat Chromosome 1. From the candidate interval, we picked the genes expressed in somites and presomitic mesoderm for candidate sequencing. The exonic sequences of the candidates were analyzed by Sanger sequencing. In the process, a missense mutation in the *Tbx6* gene was identified. Although the *Oune* mutation is localized within T-box near dimer-interface, *in vitro* experiments revealed that the *Oune* mutant proteins retain normal DNA binding ability and translational efficiency. However, we found that the mutant proteins decrease transcriptional activation potential in response to NOTCH signaling. Recently, exome sequencing of a dominant spondylocostal dysostosis family identified a stoploss mutation in *TBX6,* which leads to decrease in transcriptional activation *in vitro*. Therefore, *Oune* rats could be used as a disease model for dominant spondylocostal dysostosis and might contribute to molecular understanding of congenital vertebral malformations in humans.

P-2: Defining the Role of Mouse COPII Component SEC24C

(See abstract SO-5 in the student/postdoc session)

P-3: The *Pirc* Rat as a Model of Stochastic and Programmed Epigenetic Regulation

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The process of establishing stable epigenetic changes in the genome occurs in response to programmed developmental cues as well as environmental changes, but may also occur owing to stochastic events. We use the rat to study heritable transgenerational and developmental epigenetic changes. The *Apc*^{Pirc} rats are heterozygous for a premature truncating allele of the adenomatous polyposis coli gene. Loss of heterozygosity results in adenoma formation primarily in the colon. We examined tumor initiation through longitudinal analysis and found that the wild type allele is lost through somatic recombination in 70% of adenomas. The remaining 30% of tumors maintain their wild type copy of the gene, but are characterized as tumors through histological and immunohistological criteria. Through quantitative analysis of genomic DNA and expression of *Apc* using allele specific pyrosequencing, we have determined that half of these of tumors silence the wild type allele and only express the mutant transcript. Through longitudinal monitoring by colonoscopy, we found that this state can be dynamic but is stably maintained in most tumors over time.

We have created two congenic derivatives of *Pirc*; one on the sensitive ACI inbred background, the other on the resistant BN inbred background. We have used the congenic ACI-*Apc*^{Pirc} (N12) derivative line to examine the effect of genetic background on tumor initiation. We found that the resistant phenotype is dominant in the ACI by BN-F1. Intriguingly, the orientation of the cross has a significant impact on tumor number. When the ACI-*Apc*^{Pirc} allele is inherited maternally the male offspring develop an average of 2.9 ± 1.5 tumors, in contrast to an average of 4.9 ± 2.4 tumors when the Pirc allele is inherited paternally (Wilcoxon rank sum P=0.008). This may be due to modifier loci that are imprinted, mitochondrial effects, or differences in the maternal microbiome.

P-4: A Novel Role for an Olfactory Receptor in Pheromone Communication

(See abstract SO-7 in the student/postdoc session)

P-5: Genetic Basis of Host Susceptibility to Rift Valley Fever in Mice

Leandro Batista[*1], Satoko Tokuda[1], Tania do Valle Zaverucha[1], Laurent Guillemot[1], Dominique Simon[1], Claudia Pommerenke[2], Robert Geffers[3], Jeremy Johnson[4], Klaus Schughart[2], Marie Flamand[5], Michele Bouloy[6], Xavier Montagutelli[1], and Jean–Jacques Panthier[1]

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Introduction

The Rift Valley fever virus (RVFV) causes severe disease in both animals and humans. Host genetic determinants seem to play an important role in modelling RVF outcomes. Accordingly, we previously showed that wild-derived MBT/Pas inbred mice are highly susceptible to RVFV infection and die from acute hepatitis, recapitulating the disease in humans. BALB/cByJ inbred mice proved more resistant to infection.

Objectives

Our goal is to identify genetic factors contributing to the phenotypic variation observed between MBT/Pas and BALB/cByJ mice.

Methods

QTL analysis was performed with survival time as a trait on RVFV-infected (MBT/Pas × BALB/cByJ)F2 mice. Congenic mice carrying MBT/Pas QTLs onto the BALB/cByJ genetic background were derived and challenged with the RVFV. Prioritization of candidate genes was made using whole exome sequencing analysis.

Results

QTL interval on Chromosome 11 from MBT/Pas mice is associated with reduced survival time following RVFV infection. Congenic C.MBT-*D11Mit69* mice containing 5.5-Mb derived from MBT/Pas Chromosome 11 died significantly earlier than BALB/cByJ mice. Exome sequencing analysis predicted 35 MBT/Pas-specific damaging variants within the QTL interval.

Conclusion

Chromosome 11 from MBT/Pas mice contains genetic factor(s) that confer susceptibility to RVFV infection. Further functional studies will identify gene(s) responsible for the susceptibility of MBT/Pas mice.

P-6: Design of Validation Experiments Using the Collaborative Cross: A QTL for Sperm Curvilinear Velocity on Mouse Chromosome 2

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The Diversity Outbred (DO) and the pre-CC are transient populations in which replication of individuals with the same genotype is impossible. These populations are used to map loci responsible for a wide range of biomedical traits. A critical need in many of these studies is the ability to validate the results and to generate stable models that permit deeper phenotypic characterization. A possibility is to use the Collaborative Cross (CC) to validate the initial mapping studies and then select lines with the desired phenotype. However, it is unclear which experimental design is most robust and cost effective (in terms of time and money) to reach the proposed goals. Here we explore three scenarios (CC lines, F1 hybrids between CC lines and four-way crosses generated from these F1 mice) using a QTL for sperm curvilinear velocity (VCL) located at 72.2Mb on Chromosome 2 as a test case. This QTL was identified during a large project to dissect the genetic causes of male infertility using CC lines that became extinct during the inbreeding process (these mice are equivalent to pre-CC mice). We will compare cost, time to completion and robustness between three experimental designs: CC lines with alternative alleles at the QTL, RIX mice generated by crossing CC lines with the same functional allele at the QTL and four way crosses between these RIX mice. Allele frequency, availability of lines and reproductive performance are key factors determining the optimal experimental design.

P-7: A Novel Intronic SNP in the Myosin Heavy Polypeptide 4 Gene is Responsible for the Mini-Muscle Phenotype Characterized by Major Reduction in Hindlimb Muscle Mass

Timothy A Bell[*2], Scott Kelly[1], Sara Selitsky[2], Ryan Buus[2], Kunjie Hua[2], George Weinstock[3], Theodore Garland Jr[4], Fernando Pardo-Manuel de Villena[2], and Daniel Pomp[2]

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Replicated artificial selection for high levels of voluntary wheel running in an outbred strain of mice favored an autosomal recessive allele whose primary phenotypic effect is a 50% reduction in hindlimb muscle mass. Within the High Runner (HR) lines of mice, the numerous pleiotropic effects (*e.g.*, larger hearts, reduced total body mass and fat mass, longer hindlimb bones) of this hypothesized adaptive allele include functional characteristics that facilitate high levels of voluntary wheel running (*e.g.*, doubling of mass-specific muscle aerobic capacity, increased fatigue resistance of isolated muscles, longer hindlimb bones). Previously, we created a backcross population suitable for mapping the responsible locus. We phenotypically characterized the population and mapped the *Minimsc* locus to a 2.6-Mb interval on MMU Chr 11 a region containing ~100 known or predicted genes. Here, we present a novel strategy to identify the genetic variant causing the mini-muscle phenotype. Using high-density genotyping and whole-genome sequencing of key backcross individuals and HR mice with and without the mini-muscle mutation, from both recent and historical generations of the HR lines, we show that a SNP representing a C to T transition located in a 708 bp intron between exons 11 and 12 of the myosin, heavy polypeptide 4, skeletal muscle gene (*Myh4*; position 67,244,850 on MMU Chr 11) is responsible for the mini-muscle phenotype, $Myh4^{Minimsc}$. Using next generation sequencing, our approach can be extended to identify causative mutations arising in mouse inbred lines and thus offers a great avenue to overcome one of the most challenging steps in quantitative genetics.

P-8: 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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Our interests lie in humanizing specific regions of the mouse genome using large segments of human DNA with extents of 10s to 100s of kilobase pairs. To that end, we have undertaken a series of projects to transfer large segments of human DNA, from human BAC clones, into the orthologous region of corresponding mouse BAC clones using— (1), traditional recombineering methods; (2), recombinase-mediated cassette exchange (RCME); and (3), a novel technology that we call *trans*- or trimolecular-recombineering. In each instance the goal is to place human DNA within the larger context of homologous BAC-derived mouse arms in *E. coli*.

Completed constructs will be used to drive homologous recombination in mouse embryonic stem (ES) cells with positive selection imparted at each end of the transferred human segment using dual, recombinase recognition site-flanked, selectable marker cassettes. Properly targeted ES cells will be used to develop viable mouse strains carrying the humanized segments. Selection cassettes will be removed by crossing to mouse strains with germline expression of the appropriate recombinases. All experiments have been designed to maintain a fixed genetic background from community-standard C57BL/6 strains. Our poster presentation details the specifics of our projects and describes progress to date.

This research is supported by National Cancer Institute Grant Number 5P30CA034196-28.

P-9: The Mouse Mutant Resource: Genetic, Genomic and Phenotypic Characterization of Spontaneous Mutant Mice Arising at The Jackson Laboratory

David Bergstrom*, Laura Reinholdt, Cathleen Lutz, Michael Sasner, Stephen Murray, Stephen Rockwood, Leah Rae Donahue, and and the MMR Team

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The application of high-throughput sequencing technologies is revolutionizing the process of mutation detection. By incorporating these technologies, we in The Jackson Laboratory's Mouse Mutant Resource (JAX MMR) are extending our characterization of new spontaneous mutant strains to the point of causative gene identification. For over fifty years, the mission of the MMR (and its predecessors) has been to provide mouse models of human genetic illness to the scientific community. Through our Mouse Phenotypic Deviant Search process, atypical mice are identified from among the millions of inbred mice produced yearly at JAX and then isolated. This not only ensures genetic stability within the originating strain, but also provides a pool of potential spontaneous mutant mice for further characterization. After colonies are established, heritability is proven, and the modes of inheritance are determined, subchromosomal locations for each mutant locus are established by backcross or intercross in conjunction with SNP genotyping. In place of Sanger-based sequencing of promising candidate genes from select mutant strains, Illumina GAIIx-based high-throughput whole-exome sequencing (DNASeq), transcriptome sequencing (RNASeq), and array-comparative genome hybridization are now being incorporated to broaden the scope of mutation detection and dramatically shorten the time to causative gene identification. A general phenotypic assessment, cryopreservation of embryos or gametes, and archiving mutant DNA completes the characterization. Findings for each mutant strain are disseminated through the scientific literature or on the MMR website at http://mousemutant.jax.org/. The presentation will enumerate the many advantages of studying spontaneous mutant mice and summarize current resource offerings and metrics.

P-10: IMPReSS - International Mouse Phenotyping Resource of Standardized Screens

Andrew Blake*, Armida Di Fenza, Tanja Fiegel, Hugh Morgan, Ahmad Retha, Luis Santos, Henrik Westerberg, Steve Brown, and Ann–Marie Mallon

MRC Harwell

IMPReSS (International Mouse Phenotyping Resource of Standardized Screens), the successor of EMPReSS, contains standardized phenotyping procedures which are essential for the characterization of mouse phenotypes. Without procedure and protocol standardisation the resultant phenotype data's impact is significantly reduced, effecting reproducibility, complicating data sharing/access, and decreases potential new discovery opportunities. IMPReSS showcases definitions of the phenotyping pipelines, procedures and parameters carried out and data collected by the International Mouse Phenotyping Consortium (IMPC) - the global effort generating and characterizing a knockout mouse strain for almost every protein-coding gene in the mouse genome. IMPReSS currently consists of 11 pipelines, 140+ procedures, 3500+ measureable parameters with 4100+ associated ontology annotations. These standardized definitions allow for phenotype data to be computationally comparable and shareable with the ontological annotations permitting interspecies comparison which may help in the identification of phenotypic mouse-models of human diseases. IMPReSS is continuing to evolve to include many secondary phenotyping procedures generated from international mouse clinics working towards finalising a uniquely complete compendium of mouse phenotyping procedures. This resource is available to browse online at http://www.mousephenotype.org/impress as well as providing programmatic access to all of the data via SOAP web services.

P-11: Identification of Tumor Genetic Determinants of Response to Anthracyclines and Taxanes in an *Erbb2* Breast Cancer Mouse Model

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

P-12: A Large-Scale Mouse Mutagenesis Screen for Identifying Models of Age-Related Musculoskeletal Disease

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Bone diseases such as osteoporosis, Pagets disease and osteoarthritis are common problems in the elderly. There currently are more than 1.4 million post-menopausal women in the UK diagnosed with osteoporosis as well as a large number of undiagnosed cases. The likelihood of osteoporotic fractures is extremely high and in the UK approx. 14,000 people per year, die following hip fractures. More than 50% of people over the age of 60 are affected by osteoarthritis and it is the main cause of disability in older adults. These diseases lead to a decline in quality of life and cause significant financial burden on the NHS. The genes underlying susceptibility to age related bone impairments are poorly understood and good mouse models of ageing bone diseases are required in order to identify and study potential therapeutic targets. We are screening G3 pedigrees from the Harwell Ageing screen, using a Faxitron digital X-ray scanner for visual abnormalities, Dual Energy X-ray Analysis (DEXA) for bone mineral density and lean mass changes, grip strength, and plasma biochemistry for changes in bone metabolites. To investigate muscular function we are using grip strength as a primary screen with more detailed analysis carried out using gait analysis. We have identified models of early onset osteoporosis, skeletal abnormalities and late onset osteoarthritis. We have identified a number of pedigrees with abnormal bone parameters, including; reduced bone mineral density, osteoarthritis and synovial chondromatosis. The genetic locations of the causative mutations underlying these phenotypes have been mapped and we are currently investigating candidate genes. Among the lines of interest are: *mp107H* on a mixed genetic background including C57BL/6 and C3H (B6;C3), a line exhibiting bone abnormalities and subsequently develops osteoarthritis, and B6;C3-*mpc125H*, a line with low bone mineral density and reduced growth, we are investigating the role of *Ptpn3* in this phenotype.

P-13: A Mutation in *Atp7a* That is Sensitized in the Absence of Alpha Synuclein

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Synuclein, alpha (SNCA) has been linked to common sporadic and rare familial forms of Parkinson's disease. We are interested in the normal role of SNCA as its sequestration in inclusions during the disease course may interfere with its ability to perform some important function under stress or aging conditions. A mouse ENU mutagenesis screen of 125 pedigrees produced one mutant line that is sensitized in the absence of SNCA. This mutation was identified by a patchy coat color indicating X-linkage, and a mutation in *Atp7a* was found to segregate with the coat color phenotype. Males die within 3 weeks of birth regardless of SNCA status, while early death (at less than 35 days of age) is significantly higher in females that lack SNCA. Backcrossing from a 129S6;C57BL/6 mixed genetic background to 129S6 has lowered the rate of early death in SNCA+ females while the death rate of those that lack SNCA remains high, indicating that at least one other modifier gene exists. ATP7A is a trans-Golgi copper transporter, mutations in which cause Menkes disease in humans. Our sensitized mutation, encoding I610S, lies between the last metal binding domain and the first transmembrane domain, in a region known to be involved in appropriate subcellular localization of ATP7A. Pathology on mutant brains shows apoptosis of cortical neurons at about 2-3 weeks, with the cerebral cortex reduced in size compared to controls in males and in those females that do not survive. Localization of ATP7A to white matter tracts in striatum and corpus callosum is perturbed in the *Atp7a* mutants as compared to age-matched controls, but mislocalization is less in the presence of SNCA. To better define ATP7A subcellular localization in neurons, we are now examining primary cortical neuron cultures from wild type and mutant mice, with or without SNCA.

P-14: X Chromosome Inactivation Choice: Beyond the X Chromosome Controlling Element

(See abstract SO-2 in the student/postdoc session)

P-15: *Snai2* Participates In Different Stages Of Breast Cancer Evolution Through Its Role In Stem Cell Biology

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SNAI2 (Slug) is implicated in cancer dissemination and poor prognosis of various tumor types. It regulates

processes such as cell migration, apoptosis, or stem cell properties. SNAI2 participates in many molecular pathways essential in embryonic development, inflammation, DNA damage response, etc. Participation of SNAI2 in different pathways and physiological processes suggests it could contribute to different phases of tumor progression. To address this issue, we crossed a mouse deficient in *Snai2* with a transgenic that overexpresses the *Erbb2* (Neu) protooncogene under the MMTV promoter, both in baseline conditions and under increased oncogenic activity induced by pregnancy. *Snai2* deficiency increases tumor latency and decreases tumor number (both considered as pretumoral pathophenotypes) together with a weak defect in metastasis. After increasing the oncogenic activity of ERBB2, there was a compensation of the pretumoral pathophenotypes, but an exacerbation of the dissemination defect. These pretumoral phenotypes could be explained by a defect in basal breast proliferation and a decreased stem cell population, reflected in a lower production of mammospheres. SNAI2 was highly expressed in wildtype mammospheres. Mammary glands from MMTV-Erbb2 transgenic mice exhibited an expansion of mammospheres, but this increase did not occur in the absence of *Snai2*, and even generated less mammospheres than their non-transgenic *Snai2* wildtype counterparts. *Snai2* deficiency modified the behavior of signaling pathways downstream to *Erbb2*. Tumors from mice, completely or partially deficient in *Snai2*, had higher tumor levels of MAP kinase and AKT, both total and phosphorylated forms, especially after increasing ERBB2 oncogenic activity. Mammospheres from *Snai2* deficient mice also showed elevated levels of AKT. This study demonstrated the participation of *Snai2* in different stages of breast cancer susceptibility and progression. The mammospheres yield suggests these effects could be partly explained by the role of *Snai2* in the stem cell biology of the mammary gland.

P-16: Long-Range PCR and Next-Generation Sequecing to Identify an ENU Induced Mutation

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The chemical mutagen N-ethyl-N-nitrosourea (ENU) has become a standard tool to randomly generating complete, partial loss- or gain-of-function alleles of genes in the mouse. The major difficulties of ENU mutagenesis program are to identify the responsible base-pair change within the critical region. Strategies including SSCP, dHPLC and Sanger sequencing are routinely used, however, theses methods are labor and cost intensive. A recessive lethal family has been previously screened for all the candidate genes within the diseases interval but failed to identify the responsible causative mutation. By applying long-range PCR and deep sequencing, a non-sense mutation has been successfully identified. Our results demonstrated that by combing long-range PCR and NGS could facilitate the identification of ENU-induced mutations.

P-17: A Mutation in *Scn4a* Modifies Huntington's Disease in Mice: A Potential Novel Link Between Huntington's Disease and SCN4A Channelopathies

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Huntington's disease (HD) is an autosomal dominant, progressive, fatal, neurodegenerative disorder caused by an expanded polyglutamine tract (PolyQ) in exon one of the Huntington's gene. Up to 70% of the variance in severity and age of onset is accounted for by the number of glutamine repeats. However, the rest of the variance is accounted for by environmental and genetic factors (modifiers). Genetic modifiers represent potential targets for the development of new therapeutic approaches to neurodegenerative disease. In order to identify modifiers modulating the HD phenotype, we have been pursuing a genetic screen using N-ethyl-N-nitrosourea (ENU) random mutagenesis on a mouse model of HD. We injected BALB/c males, crossed them to Tg(HD82Gln)81Dbo (HD mouse model) transgenic carrier females and assessed onset and progression of the disease in HD F1s using a variety of simple behavioural analyses and weight measures. Analysis of progeny to date has identified several mice lines carrying inherited dominant modifiers. The gene responsible for one of the HD enhancer lines has been identified. Mice carry a novel mutation in the mouse skeletal muscle specific voltage-gated sodium channel (official allele symbol *Scn4a*^{m1Aaa}). The *Scn4a*^{m1Aaa} male mice carrying the HD transgene (HD; *Scn4a*^{m1Aaa}/+) enhance the overall HD disease phenotype. Heterozygous mice also have relevant phenotypes independent of the HD transgene. Mutations in *SCN4A* are causative of periodic paralysis (PP) and nondystrophic myotonias. *Scn4a*^{m1Aaa} mice have myotonia and hind-limb paralytic attacks, modelling all aspects of *SCN4A* channelopathies. Moreover, a family carried a novel *SCN4A* mutation, equivalent to the mouse mutation, was identified. Interestingly, while the *Scn4a*^{m1Aaa} mutation enhances the overall HD phenotype, the HD transgene also sensitized *Scn4a*^{m1Aaa} mice to have more hind-limb paralytic attacks. We have therefore uncovered a potential downstream interaction between the two pathologies, providing new insights into the pathological mechanisms of both diseases.

P-18: Germline Variation Modulates Susceptibility to Aggressive Disease Development in a Mouse Model of Prostate Tumorigenesis

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Although prostate cancer is common, with over 238,000 new cases being diagnosed in the US in 2012, it typically runs an indolent course with most men succumbing to unrelated disease processes. This is reflected in the low prostate cancer-specific mortality, with approximately 29,000 men succumbing in the same period. It is of critical importance to identify modifiers that increase susceptibility to aggressive disease to allow physicians to more accurately identify men at risk of fatal disease forms. The goal of this work is to map prostate tumor progression and metastasis modifier loci mapping using the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of aggressive neuroendocrine prostate carcinoma. We hypothesize that germline variation influences tumor progression and metastasis in prostate cancer.

The effect of germline variation in TRAMP mice was investigated by crossing it to Collaborative Cross progenitor strains and quantifying tumor progression and metastasis in transgene-positive F1 males. Those strains with the greatest phenotypic variation from the wildtype TRAMP C57BL/6J mice were chosen for modifier mapping using an F2 intercross approach. F2 mice were genotyped using a linkage panel consisting of 1,449 SNPs and modifier loci analyzed using j/qtl. The greatest number of loci achieving genomewide significance were observed in the TRAMPxNOD/ShiLtJ F2 cross (n=232). Modifier loci associated with primary tumor growth were observed on Chromosomes 4, 7 and 8. Additionally, loci associated with metastasis were observed on Chromosomes 1, 11, 13 and 17.

We have therefore identified multiple loci associated with aggressive disease development in a mouse model of prostate cancer. Candidate gene identification is ongoing, and focuses upon characterizing cis-eQTLs in TRAMPxNOD/ShiLtJ F2 primary tumors. Additionally, we are performing high resolution modifier mapping in TRAMPxJ:DO F1 mice. Our eventual aim is to confirm the relevance of candidate genes identified in the TRAMP mouse in human association cohorts.

P-19: ENU Mutagenesis Identifies Signalling Pathways

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We are conducting a three generation screen for recessive ENU-induced mutations that cause eye and vision defects in the mouse. Mice homozygous for either the *goya* or the *ceo* mutation are born with their eyes open. In the case of *goya*, mutant adult eyes are highly variable, ranging in pathology from apparently normal to bulging to microphthalmic. Irrespective of eye phenotype *goya* mice fail to respond in a visual function assay. Homozygotes for *ceo* have small cloudy eyes and rarely survive past weaning. Both mutations map to genes known to cause defects in eyelid formation when deficient. For *goya*, we found a splice site mutation in the serine/threonine kinase *Map3k1* and for *ceo* there is a missense mutation in the shedding factor *Adam17* that affects a crucial amino acid in the catalytic site of the protein. Mutations in *Tgfa* and its receptor *Egfr* also cause an eyes open at birth phenotype suggesting that all four genes act in the same pathway. To investigate this we combined *goya* and *ceo*. Mice heterozygous for either mutation have normal eyes, whilst those heterozygous for both have eye defects. This genetic interaction strongly implies that *Adam17* and *Map3k1* are in the same developmental pathway for eyelid formation. Mice heterozygous for *goya* and heterozygous for an *Adam17* knock-out have normal eyes indicating that the presence of mutant ADAM17 protein in *ceo* heterozygotes is more detrimental than reducing the amount of protein by half.

P-20: Annotating Non-Coding Genes in Mouse: How Conserved Are These Genes?

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The HAVANA group is tasked with annotating all types of genes - coding, non-coding and pseudogenes - and gene related genomic features in the human, mouse and zebrafish genomes. Comprehensive, feature rich manual annotation, performed using robust in-house tools and software, is supplemented with automated Ensembl gene predictions. The combined output constitutes the GENCODE gene set, which was a fundamental component of the globally implemented ENCODE (Encyclopedia Of DNA Elements) project. Non-coding genes are annotated and updated on a continuous basis using guidelines that take account of the latest advances in the experimental research of non-coding genes as well as the latest sequencing technologies. The mouse will continue to be the best model for the study of human genes, which will ultimately lead to practical applications for human disease prevention and health. Therefore we need to fully and comprehensively annotate the mouse genes and study their conservation and syntenic relationship with human genes. While conservation has been fully established in many protein coding genes, it has not been shown in the case of the non-coding genes. Here we will describe examples of non-coding genes, both with and without conservation, and discuss the most likely role of conservation (or lack thereof) of non-coding genes.

P-22: CrePortal.org, A Critical Resource for Conditional Mutagenesis

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A challenge to effectively using the large collection of conditional-ready loxP flanked alleles generated by the International Mouse Knockout Consortium (IKMC, www.knockoutmouse.org) is the selection of appropriate cre-recombinase mice with which to mate the IKMC allele-bearing mice to generate specific spatial and temporal knockouts.

The CrePortal (www.creportal.org) provides critical data about cre constructs, including the driver, whether recombinase activity is inducible (and by what), strain availability through public repositories, and publications describing conditional mutagenesis done using each cre allele. Histological images, annotated with activity patterns, anatomical structures, and ages assayed defining cre specificity can assist selection of optimal cre-bearing strains for specific experiments. Whole slide viewing is available for some cre lines, notably submitted by JAX and from the Allen Brain Institute. Access to cre specificity data are critical in determining the best cre-bearing strains for experiments, not only for knowledge about activity at the desired target (and its time/space distribution), but also in considering 'off-target' activity that may complicate interpretation of observed phenotypes.

Important new features have been implemented in the CrePortal in response to user comments. These include: 1) the ability to search for cre activity by specific tissue or structure, such as 'left ventricle cardiac muscle' (formerly only searches by anatomical system were available, e.g. cardiovascular system); 2) a summary matrix of cre activity in structures/tissues assayed vs. age (e.g., for left ventricle cardiac muscle, one can visualize its activity by age distribution; and also note off-target embryonic expression in liver and pharynx); 3) a 'Your Observations Welcome' section for contributions of laboratory experience with particular cre mouse strains, as many 'facts' about performance of cre lines remain anecdotal; and 4) a submission form for data and image files on new cre strains, or additional data on existing strains.

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P-23: *Lman1* Deficient Mice Reveal New Insights Into Coagulation Factor V and VIII Secretion

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

P-24: Host Genetic Contributions Underlying Respiratory Function and Distress Following Acute Viral Infection.

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Infection with respiratory pathogens usually results in clinical manifestations of lung damage and respiratory distress, such as acute respiratory distress syndrome (ARDS). In contrast, most *in vivo* experimental models of respiratory pathogens focus on behavioral or condition-based (e.g. weight loss) metrics of clinical disease. Here we describe the use of whole-body plethysmography to measure respiratory function of mice infected with either Influenza A Virus (IAV) or Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). Both pathogens cause acute disease in the C57BL/6J mouse model of infection, and there is evidence of long-term lung remodeling following recovery from infection with these pathogens. We find that multiple aspects of the respiratory response are changed in response to infection, that these changes are dynamic processes during the infection, and that these changes in respiratory responses are unique to each pathogen. We have also begun investigating the role of host genetic variation on these respiratory responses to acute viral infection by utilizing Collaborative Cross Recombinant Inbred Intercross (CC-RIX) animals infected with these two pathogens, with a particular focus on host genome regions previously shown to play a role in inflammatory cell infiltration, activation, and lung pathology following SARS-CoV or IAV infection within incipient lines of the Collaborative Cross.

P-25: A Multi-allelic Clustering Approach for Improving Genotype Calling

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In DNA microarray analysis, distinct alleles appear as separate clusters in probe intensity space. However, the positions and number of clusters can vary widely from marker to marker. Classic genotype calling algorithms have adopted a common parameterized cluster model that is shared among all markers. We propose one unsupervised and one semi-supervised alternative clustering algorithms that allow for markers with widely varying cluster shapes and numbers.

We demonstrate our methods on a newly developed murine genotyping array called MegaMUGA, which includes 77.8K SNP markers and is built on the Illumina Infinium platform. The method is optimized to genotype samples

that are mosaics of eight inbred laboratory mouse strains of the Collaborative Cross (CC), their 28 F1 combinations, and Diversity Outcross (DO) populations.

For unsupervised algorithm, we apply a Bayesian approach based on a nonparametric prior. Using a Dirichlet process with an unknown number of components, we infer a probabilistic Gaussian mixture model. Along with the model, our algorithm exploits a novel two-stage Gibbs sampling approach for posterior inference. In contrast to Dirichlet process, we propose another semi-supervised algorithm, inferring clusters from probe intensities by applying Hotelling T-square test. The algorithm first creates a reference-clustering model with founder replicates only, and then infers clusters of F1 combinations according to the result of reference model.

Finally, we choose the best representative one between two algorithms as the clustering result for each marker and provide the corresponding mean and covariance of each cluster for further usage. The demonstration shows that our algorithms are able to observe substantial number of multi-allelic markers and eliminate the errors in markers with atypical intensity patterns.

P-26: Inferring Founder Probabilities in Admixed Animals using Genotyping Intensities

(See abstract SO-8 in the student/postdoc session)

P-27: Phenotypic Characterization of Mutant Mouse Lines in the German Mouse Clinic

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The German Mouse Clinic (GMC) offers a systemic, standardized phenotype analysis of mutant mouse lines by scientists from various disciplines. The phenotypic screens focus on the areas of allergy, behavior, bone and cartilage, cardiovascular diseases, clinical chemistry, eye development and vision, energy metabolism, immunology, lung function, neurology, nociception, molecular phenotyping, steroid-metabolism, and pathology. The standardized phenotyping procedures are under supervision of a specialized quality manager, and we are in the process of preparing the certification of our phenotyping procedures. Within the frame of Infrafrontier the German Mouse Clinic contributes to the phenotyping efforts of the International Mouse Phenotyping Consortium (IMPC). In addition, the GMC is open for collaborations, and offers its phenotyping capacities to the scientific community as a scientific collaboration.

In GMC II we established environmental challenge platforms for the analysis of genome-environmental interactions, and currently we are in process to develop GMC III for the analysis of drug and compound actions. Another focus of the GMC is in the development of new technologies for mouse phenotyping like for example infra-red based analysis or new imaging techniques.

P-28: Functionality of Amino Acid Substitutions and Other Single Nucleotide Variations in the Mouse

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Single nucleotide substitutions (SNVs) cause various mutations in mammalian genome, for instance, missense mutations, nonsense, frameshift, splicing and many other mutations including noncoding mutations. SNVs are also considered to be the cause of various human diseases due to the deleterious effect of the gene and genome function. We have been screening SNVs in the RIKEN Mutant Mouse Library to provide “next-generation gene targeting” by which user may obtain allelic series of SNVs in any target genes. We have been identifying about 10 SNVs per requested target gene. We also found and reported that roughly 70%, 10% and 20% of the SNVs in ORF are missense, KO-equivalent and synonymous mutations in the mouse genome. One of the key questions is what a part of the missense and other mutations may have the functional changes. Our users have already reported many functionality changes of missense mutations, e.g., in *Disc1* [1], *Nat1* [2], *Srr* [3], *Rtn4r* [4], *Bcl11b* [5] and *Fam105b* (Gumby) [6]. This question also extends to the functionality of noncoding SNVs. One noncoding SNV was indeed identified with the long-distance cis-regulatory change due to the T to C transition [7]. We have so far revived more than 100 mutant mouse strains carrying an SNV in a target genomic sequence and discuss the functionality of the SNVs found in RIKEN Mutant Mouse Library.

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3. Labrie et al. Hum Mol Genet 18:3227-3243, 2009.

4. Lazar et al. Behav Brain Res 224:73-79, 2011.

5. Katsuragi et al. Mech Dev, 2013, in press.

6. Rivkin et al. Nature 498:318-324, 2013.

7. Masuya et al. Genomics 89:207-214, 2007.

P-29: Systems Pharmacogenomics Approach Identifies Synergistic Molecular Action of Combined MTOR/HDAC Inhibition on MYC

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

P-31: The European Mouse Mutant Archive - EMMA

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The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. At present EMMA holds more than 3200 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 EUCOMM ES cell resource.

The EMMA network is comprised of 16 partners from 13 different countries who operate as the primary mouse repository in Europe. EMMA is funded by the partner institutions, national research programmes and by the European Commission's FP7 Capacities Specific Programme. EMMA is part of the INFRAFRONTIER Research Infrastructure that coordinates projects and services in the field of mouse disease models in Europe (www.infrafrontier.eu).

In addition to the core services of archiving and distribution of mutant mouse lines, the EMMA consortium can generate germ-free (axenic) mice for its customers and also hosts courses in cryopreservation, to promote the use and dissemination of frozen embryos and spermatozoa. Dissemination of knowledge is further fostered by a dedicated resource database. EMMA's technology development programme focuses on improving sperm cryopreservation methods and the implementation of laser-assisted IVF and ICSI protocols.

All applications for archiving and requests for mutant mouse strains are submitted online at www.emmanet.org. Mouse strains submitted for archiving are evaluated by EMMA's external scientific committee. Once approval has been granted depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or spermatozoa cryopreservation. Strains held under the EMMA umbrella can be provided as frozen materials or re-derived and shipped as live mice depending on the customer's needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery.

P-32: SINEUPs: Novel Antisense ncRNA Can Enhance Protein Translation

(See abstract SO-1 in the student/postdoc session)

P-33: The Metabolic Syndrome in Closely Related Berlin Fat Mouse Inbred Lines

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The Berlin Fat Mouse Inbred lines BFMI861 S1 and S2 were generated by selection and inbreeding through mating of different full sibs in generation 23 and subsequent continuation of inbreeding for additional 43 generations.

We characterized both inbred lines with respect to the metabolic syndrome, which is considered as a complex of risk factors for later diseases, in particular diabetes mellitus type II. Features of the metabolic syndrome are obesity, insulin resistance and dyslipidemia. We measured body composition and intramuscular fat content by MRI and NMR, respectively. Insulin sensitivity was evaluated by the intraperitoneal and oral glucose tolerance (IPGTT, OGTT, respectively) tests and intraperitoneal insulin tolerance (IPITT) test. Dyslipidemia was assessed through an oral lipid tolerance test (OLTT) and measurement of serum lipids.

Both lines develop juvenile obesity and have a fat content of 35.5% (S1) and 28.5% (S2) at 20 weeks. The lines differ extremely in their ability to take up glucose into peripheral tissues. Insulin sensitivity is markedly reduced in BFMI861 S1 mice. These mice have no capacity to clear blood glucose values within two hours, albeit insulin levels are high. Furthermore, the metabolic flexibility in using carbohydrates or fat is reduced in BFMI861 S1 mice. Triglycerides in the serum were 1.5 times as high in S1 compared to S2 mice, and nonesterified fatty acids were 1.2 times increased. While the line BFMI861 S1 has major disturbances in the insulin signaling cascade and switching between substrates, line BFMI861 S2 responds metabolically healthy. Genotyping using the Mouse Diversity SNP Array revealed polymorphisms in genes of the insulin signaling pathway.

Since the two lines differ extremely in insulin sensitivity and serum lipids, and have relatively few genetic differences, a cross-breeding experiment between both lines could identify chromosomal regions with impact on insulin sensitivity and diagnostic factors for the metabolic syndrome.

P-34: Introduction of a Human Epilepsy Mutation into the Mouse Sodium Channel Gene *Scn8a* Using TALEN Technology

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Targeted mutation of mouse genes by homologous recombination in ES cells is labor-intensive and costly. More efficient generation of mouse models expressing human disease mutations may be possible using TALEN technology. We evaluated the use of TALENs to target the missense mutation Asn1768Asp into the neuronal sodium channel gene *Scn8a* (Nav1.6). We previously identified this de novo mutation in a child with epileptic encephalopathy (Veeramah et al, Am. J. Hum. Genet. 2012). Five pairs of TALEN-Fok1 endonucleases were designed and tested in transfected cells by PNA Bio, Inc. (Thousand Oaks, CA). Polyadenylated and 5' capped mRNAs encoding the most active pair of TALENs were co-injected into 350 fertilized mouse eggs, together with a circular targeting vector containing 3.8 kb of mouse genomic DNA carrying the missense mutation. Among the 67 potential founders born, 5 heterozygous carriers of the mutation were identified by Southern blotting. An additional 24 offspring carried small indels near the target site, resulting in several null alleles of *Scn8a* and one novel in-frame deletion of a single amino acid. Since *Scn8a* is a member of a multigene family, we screened for off-target mutations in two family members that differ from *Scn8a* by 1 to 2 bp within each TALEN binding site. Seven mice with heterozygous off-target mutations were identified. The targeted *Scn8a* mutation has been transmitted to the next generation, and analysis of the epilepsy phenotype in carriers is in progress. The use of TALENs permitted the rapid generation of this mouse model. Substitution of a synthetic oligonucleotide for the targeting vector may further increase efficiency. This technology will facilitate the functional evaluation of selected variants discovered by patient exome sequencing. (Supported by NIH R01 NS34509.)

P-35: Comparative mouse and human mRNA investigation suggest novel candidate genes involved in the pathogenesis of Major Depressive Disorder

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

P-36: Identification of Skin Tumor Modifier Genes in Japanese Wild Derived Inbred Mouse Strain, MSM/Ms.

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MSM/Ms is an inbred mouse strain derived from a Japanese wild mouse, *Mus musculus molossinus*. In this study, we showed that MSM/Ms exhibit dominant resistance when crossed with susceptible FVB/N mice and subjected to the two stage skin carcinogenesis protocol using 7, 12-dimethylbenz(a)anthracene (DMBA)/ 12-O-tetradecanoylphorbol-13-acetate (TPA). A series of F1 backcross mice were generated by crossing *Trp53*+/+ or *Trp53*+/- F1 (FVB/N x MSM/Ms) males with FVB/N female mice. These generated 228 backcross animals, approximately half of which were *Trp53*+/-, enabling us to search for *Trp53*-dependent skin tumor modifier genes. Highly significant linkage for papilloma multiplicity was found on Chromosome 7. Furthermore, in order to identify stage-dependent linkage loci we classified tumors into three categories (< 2 mm, 2-6 mm and > 6 mm),

and did linkage analysis. The same locus on Chromosome 7 showed strong linkage in groups with < 2 mm or 2-6 mm papillomas. No linkage was detected on Chromosome 7 to papillomas > 6 mm, but a different locus on Chromosome 4 showed strong linkage both to papillomas > 6 mm as well as to carcinomas. This locus, which maps near the *Cdkn2a* gene, was entirely *Trp53*-dependent, and was not seen in *Trp53*+/- backcross animals. These results clearly suggest distinct loci regulate each stage of tumorigenesis, some of which are *Trp53*-dependent. To narrow down the region, we generated multiple congenic strains segregating Chromosomes 7 and 4, and have confirmed the skin tumor susceptibility locus within genetic distance of about 6 cM on proximal Chromosome 7. Now, we are trying to generate congenic lines having the smaller region, which together with bioinformatics analysis will facilitate the following gene identification step.

P-37: A Nonsense Mutation in the Dystonin Gene Causes Hereditary Sensory and Autonomic Neuropathies in Mice

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Hereditary sensory and autonomic neuropathies (HSANs) are a clinically and genetically heterogeneous group of peripheral nervous system disorders characterized by progressive degeneration, predominantly of the sensory and autonomic neurons, and cause prominent sensory loss and ulcerative mutilations in combination with variable autonomic and motor disturbances. This disorder appears in several forms and causative mutations have been identified in 12 genes; however, the molecular mechanisms underlying this disorder are not known. In this study, we identified a novel recessive mutation, that is, a torsion dystonia (*tdt*) mutation that arose spontaneously in the JF1/Ms-*Ednrb*$^{s+Ms}$/Ms strain; in this strain, the homozygotes develop severe movement disorders, including hypertonic musculature of the extremities, writhing of the trunk, and inability to walk, and then die at 3–4 weeks of age. To identify the *tdt* mutation, we used a positional cloning approach. The mutation was mapped to an approximately 1.5-Mb interval on Chromosome 1; this interval contains the dystonin (*Dst*) gene (also known as bullous pemphigoid antigen 1, *Bpag1*), which is responsible for HSAN6 in humans. Using sequence analysis, we found that the responsible mutation was a 412 C ↔ T transition leading to the substitution of glutamic acid by a nonsense codon. This mutation is predicted to cause decreased expression of *Dst* because some nonsense mutations lead to functional inactivation through rapid mRNA degradation. Therefore, we performed RT-PCR analysis to examine the effect of the *tdt* mutation on *Dst* expression and confirm the reduction in *Dst* mRNA expression in the *tdt* mutant. The results suggest that the *tdt* mutant is a new mouse model for human HSAN6.

P-38: Determining the Role of Innate Immunity in Mice Resistant to Rift Valley Fever

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

P-39: Analysis of the Host Response to Influenza A Virus Infection in the Collaborative Cross Founder Strains

(See abstract SO-9 in the student/postdoc session)

P-40: Exploring Regulatory Networks and Interactions of *Mir96* in the Developing Inner Ear

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

P-41: The Rare and Orphan Disease Center at The Jackson Laboratory

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Objective: The Rare and Orphan Disease Center at The Jackson Laboratory focuses on bringing together global mouse resources and the expertise of research scientists to develop and distribute mouse models which better recapitulate human disease. The Center's Mission is to a) join with experts from around the world, including foundations, researchers and pharmaceutical developers for innovative partnerships to ensure we collectively get the most out of mouse models of rare diseases; b) engineer new solutions by creating, refining and expanding the variety of rare disease preclinical models available to the scientific community; c) accelerate drug discovery and efficacy testing via worldwide distribution of over 7,000 mouse strains.

Methods: We genetically engineer new mouse models, from construct creation and microinjection, to evaluation and characterization. We enhance existing models through phenotypic evaluation, quality assurance and genetic background standardization. We distribute validated preclinical mouse models for drug discovery to the worldwide biomedical community.

Results: Ongoing rare disease research projects involve partnerships with foundations, companies and principle investigators. To date, our primary focus has been on model creation and genetic standardization for rare neurological disorders, such as spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease), Friedreich's ataxia, and Duchenne muscular dystrophy (DMD). We currently distribute over 1,800 for the study of rare and orphan diseases and translational models harboring patients' mutations.

Conclusions: With over 7,000 human rare diseases and approximately 350 million people affected worldwide, to develop treatments or cures, partnerships and collaborations across diverse areas of expertise are needed. We are interested in partnering with new groups across all therapeutic areas of research. Visit our website at www.jax.org/rare to learn more.

P-42: New Computational Method to Identify Epigenetics Markers for Colorectal Cancer Diagnosis

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Background: DNA methylation is a well-known mechanism for transcriptional silencing. When aberrant, it can be used in diagnostics of various diseases. We developed a novel computational method to identify a new set of methylation-based biomarkers for colorectal cancer (CRC) diagnosis.

Observations: Our new computational method for colorectal cancer cancer (CRC) diagnosis, involves the following steps: 1/ screening for genes methylated in CRC tissue using PubMed (more than 2000 entries); 2/ inferring methylation values of these genes from their tissue expression data available in GEO-NCBI; 3/ selection of a panel of significantly hyper-methylated genes in CRC; 4/ testing the panel's combined predictive performance with a thresholding algorithm. We also evaluated predictive performance in the presence of resistance to noise based on the threshold. Finally, we observed the general behavior of methylation versus (normalized) expression on ENCODE data by averaging expression in bins of 10% methylation. 25 genes were found statistically hypermethylated in CRC ($p<10^{-5}$) and discriminate 100% of the CRC patients from the normal, even with increasing the error margin from 5% to 33%. Predictive performance tests identify 14 markers that, when used in combination, provide 100% of sensitivity and specificity. The ENCODE data in the high methylation/low expression segment supports our gene panel.

Conclusions: Our finding proposes a composite biomarker for the diagnosis of CRC and our strategy could be extended to identify composite methylation-based biomarkers for other cancers.

P-43: Nomenclature for Many-to-Many Homologene Sets

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Mouse Genome Informatics (MGI, www.informatics.jax.org) maintains the international authoritative resource for identity and names of mouse genes, genetic markers, alleles, chromosome aberrations, genomic features, and strains. Recently MGI implemented a new representation of gene sets containing multiple paralogs (in addition to orthologs). This change allows MGI to represent 649 additional HomoloGene data sets, 3.2% of the total containing mouse protein coding genes.

This expansion affects nomenclature of genes with poorly informative symbols and names. For example, Homologene set ID 81946 contains one human gene, three mouse genes and one rat gene in the *Llph* family. One mouse paralog has the *Llph* symbol, the other two are named as gene models. *Llph* will be modified to indicate it is a member of a multigene family and *Gm12952* and *Gm14535* will be given new *Llph* family symbols.

Additionally, MGI can now provide more accurate nomenclature for other gene sets. For example, Homologene set ID 78045 contains two human genes, seven mouse genes and two rat genes in the *Spin2* family. MGI already has a gene with symbol *Spin2*, not included in this set. Examination of GeneTree shows that all the genes are closely related. Therefore, *Spin2* should be modified and other paralogs given the *Spin2* root symbol.

The mouse and human nomenclature committees strive to use the same symbols and names for orthologs. More complete genomic sequence data and many-to-many homologs result in a nomenclature conundrum. Ideally, there should be a way to indicate that genes share a many-to-many relationship instead of the typical one-to-one. In very large gene families, such as the zinc finger proteins and olfactory receptor families, human and mouse root symbols are similar but not identical. This system might be adopted for nomenclature for these smaller gene sets. In any case, such gene sets will require ongoing evaluation.

P-44: Generation of Knockdown Mice Using Pronuclear Injection-Based Targeted Transgenesis: Advantages and Limitations

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RNAi-mediated gene silencing is a powerful strategy to study gene function. However, artificial microRNA transgenic mice generated via traditional pronuclear injection frequently exhibit highly variable knockdown efficiency due to the random transgene integration with multiple copies. This study generated knockdown mice using pronuclear injection-based targeted transgenesis (PITT) method that enables the integration of a single copy transgene into *Gt(ROSA)26Sor* using cre-loxP site-specific recombination. We first constructed artificial microRNA expression cassettes by introducing artificial microRNA against the EGFP and tyrosinase genes into the 3′UTR region of the fluorescent reporter expression cassette. As for the EGFP knockdown, three transgenic lines containing this microRNA expression cassette were obtained by the PITT method, and each of them was crossed with the previously developed EGFP transgenic mouse. In the double transgenic mice, the intensities of EGFP fluorescence and EGFP mRNA levels were reduced to an identical level among all three lines, indicating that knockdown levels by microRNAs are highly reproducible in the mice generated by PITT method. We also confirmed that tyrosinase knockdown mice exhibited light coat color compared with the wildtype indicating that PITT method is applicable to the *in vivo* knockdown of the endogenous gene. However, homozygote knockdown mice were hardly recovered for both constructs, suggesting the toxicity of overexpression of artificial microRNA.

P-45: Compound Heterozygosity of the Functionally Null *Cdh23$^{v\text{-}ngt}$* and Hypomorphic *Cdh23^{ahl}* Alleles Leads to Early-Onset Progressive Hearing Loss in Mice

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The waltzer (*v*) mouse mutant harbors a mutation in Cadherin 23 (*Cdh23*) and is a model for Usher syndrome type 1D, which is characterized by congenital deafness, vestibular dysfunction and prepubertal onset of progressive retinitis pigmentosa. In mice, functionally null *Cdh23* mutations affect stereociliary morphogenesis and the polarity of both cochlear and vestibular hair cells. In contrast, the murine *Cdh23^{ahl}* allele, which harbors a hypomorphic mutation, causes an increase in susceptibility to age-related hearing loss in many inbred strains. We produced congenic mice by crossing mice carrying the waltzer Niigata (*Cdh23$^{v\text{-}ngt}$*) null allele with mice carrying the hypomorphic *Cdh23^{ahl}* allele on the C57BL/6J background, and we then analyzed the animals' balance and hearing phenotypes. Although the *Cdh23$^{v\text{-}ngt}$/Chd23^{ahl}* compound heterozygous mice exhibited normal vestibular function, their hearing ability was abnormal: the mice exhibited higher thresholds of auditory brainstem response (ABR) and rapid age-dependent elevation of ABR thresholds compared with *Cdh23^{ahl}* homozygous mice. We found that the stereocilia developed normally but were progressively disrupted in *Cdh23$^{v\text{-}ngt}$/Chd23^{ahl}* mice. In hair cells, CDH23 localizes to the tip links of stereocilia, which are thought to gate the mechanoelectrical transduction channels in hair cells. We hypothesize that the reduction of *Cdh23* gene dosage in *Cdh23$^{v\text{-}ngt}$/Chd23^{ahl}* mice leads to the degeneration of stereocilia, which consequently reduces tip link tension. These findings indicate that CDH23 plays an important role in the maintenance of tip links during the aging process.

P-46: Starting at the Ends: High-Resolution Sex-Specific Linkage Maps of the Mouse Indicate that Recombination in Male Germline Progresses from Telomeres to Centromeres

(See abstract SO-4 in the student/postdoc session)

P-47: The Collaborative Cross Mouse Population for Dissecting Host Susceptibility to Mixed Infection Inducing Alveolar Bone Loss

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Periodontitis is an infectious disease of the supporting tissues of the teeth that influenced by host genotype and environmental factors. Here, we initiated study aimed to define the phenotypic response (alveolar bone loss) of over 100 inbred lines of the (CC) mouse population to periodontal bacterial challenge using an experimental periodontitis model, and subsequently perform genome-wide search for quantitative trait loci (QTL) associated with host susceptibility to the diseases. In total, 1021 mice of 111 lines were characterized. (On average, 5 mice per line for infection and 5 for control). Briefly, infected mice were orally co-challenged with *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. The infection was repeated three times at 2 day intervals. 42 days following the final infection, the maxillary jaws were harvested and alveolar bone volume was quantified using microCT. Based on ANOVA results, some lines out of the tested CC population showed significant decrease in the bone volume after the co infection and considered to be susceptible lines ($P \leq 0.05$), while the remaining lines were resistant to alveolar bone loss. Basic bone volume of CC lines was significantly different ($P \leq 0.05$). Sex effect of

the mice on bone volume of control and infected mice was tested across the different lines, and found not to be significant. Broad-sense heritability of the bone volume trait of naïve mice and bone loss volume of mice due to infections was estimated and found to be 0.4 and 0..2, respectively. Initial QTL mapping was conducted on 23 lines using HAPPY software, and number of QTL associated with naïve, residual (differences between naïve and infected) and proportion (residual/naïve) bone volumes, were mapped at different chromosomal regions.. These results confirmed that host susceptibility to periodontitis is a complex trait and controlled by multiple genetic factors and the CC population is a powerful tool for dissecting this trait.

P-48: Assessment of Comparative Functional Annotation Propagation in Mouse

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The Mouse Genome Informatics (MGI) project has long exploited orthologous mammalian relationships to infer function of mouse genes from experimentally determined knowledge about human and rat genes. Recently, MGI implemented a M:N orthology paradigm to better reflect current understanding about the relationships between genes of these three organisms. Although one-to-one orthology assertions between mouse/human/rat genes still holds for 90% of protein-coding genes, MGI can now more clearly represent cases such as *Serpina1a* class where phylogenetic analysis shows 5 mouse genes, 1human gene, and 1 rat gene in the same orthology class.

The Gene Ontology (GO) is widely used to annotate molecular attributes of genes and gene products. GO supports the use of shared semantics and standards for functional annotation, facilitating comparative genomics endeavors that will lead to a better understanding of human biology and disease. Annotations that have been curated from the literature by domain experts are considered the most valuable component of this effort, but manual curation is very labor intensive compared with semi-automated methods for assignment of functional annotation. MGI's use of new orthology sets has led to the refinement of rules for semi-automated annotation propagation.

Since genes that share close evolutionary relationships are likely to function in similar ways, many applications leverage phylogenetic relationships to propagate functional annotation from related genes. This process involves two distinct steps: (1) the assertion of orthology, and, (2) since function is not necessarily conserved across speciation and gene duplication events, the determination that annotation propagation is sound.

We assess both the quantity and quality of various methods of automated propagation of functional annotations. As more genomes are available, automated methods for annotation propagation homology sets will become more important. We hope this work will contribute to maintaining the high quality of functional annotation sets.

P-49: Cardiac Phenotypes Arising From the Harwell Aging Screen

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As part of the Harwell Aging Screen, an ENU-base phenotype-driven screen, we have identified a number of lines with suspected or confirmed cardiac phenotypes. Several of these have been mapped and are under investigation. An early onset phenotype was observed in *mpc91H* mutant mice which we presume to be a model of sudden death. Mice exhibit little outward sign of ill-health but homozygotes rarely live beyond 7 weeks. We have identified a subtle behaviour indicative of ill health. Homozygote mice do seem to exhibit a small, but significant, increase in QRS duration and there are clinical chemistry abnormalities associated with the phenotype. We are investigating whether these are biomarkers of disease or secondary to the primary phenotype. The phenotype has been mapped to Chromosome 7 and we are currently analysing coding and non-coding mutations identified in the region, including a mutation in *Bcat2*, a mitochondrial protein. A second line carrying the *mpc178H* mutation was identified as developing hypertrophic cardiomyopathy, with a significant impact on health at around 6 months of age. This has been mapped and we have identified a strong candidate within the region, *Smarca4*, which has previously associated with this disease. We are carrying out further analysis to confirm this gene as a candidate. Other late onset, degenerative cardiac phenotypes have been identified and are being mapped.

We are about to begin an ECG screen of all mice entering the ageing screen to identify abnormalities and determine the long term consequences of these mutations.

P-50: Analysis of Directional Mutation Pressure in the Mouse Genome

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We mapped 3,380 ENU-induced mutations to the mouse genome and computed GC content values of their flanking genomic regions. The distributions of the GC content values corresponding to mutational directions (AT->GC and GC->AT) suggest two distinctive phenomena: (1) mutations occurred in AT rich regions have a tendency towards AT rich; (2) mutations occurred in GC rich regions have a tendency towards GC rich. These mean that there exist two kinds of opposite mutation patterns bounded by a certain GC content value (or a GC content range). This discovery is consistent with Sueoka's classical idea on the GC content evolution [1]. Considering that the ENU-induced mutations have a widely ranged spectrum, it is plausible that their mutation patterns fairly mimic spontaneous mutation spectra but in an exaggerated way: i.e., with higher mutation rates across the spectra. Since we observed purely raw mutation patterns of the "first" generation (G1), it is probable that we could detect an essential signal(s), excluding unwanted noises that might conceal meaningful but subtle mutation patterns. Furthermore, since our ENU-induced mutations in G1 heterozygotes are considered to be virtually neutral, no matter where they fell, we could estimate genome-level evolution in an ideal way. We report these purely "raw"-level mutation patterns, which may explain the queer genome-level spatial structure of GC content, called isochore [2].

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P-51: MegaMUGA, a Second-Generation Medium-Density Custom Designed Genotyping Array for the House Mouse

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We have designed and developed a new array for laboratory mice that addresses the competing interests of higher density and lower price. The array known as MegaMUGA builds upon the success of MUGA (Mouse Universal Genotyping Array) while increasing the genotyping density by 10 fold and addressing specific shortcomings. The array design and probe content was largely driven by the needs of the Collaborative Cross and Diversity Outbred populations and thus the SNPs were selected to be maximally informative in the eight founder inbred strains of these two resources. In addition, we selected SNPs that provided diagnostic information to discriminate between *Mus musculus* subspecies and *Mus* species. In contrast with the first generation MUGA array, MegaMUGA includes reliable markers for the mitochondria, chromosome Y and pseudoautosomal region. We also included markers that discriminate between closely related strains, with particular emphasis in discrimination between sister strains of the popular C57BL/6 inbred strain. In addition to probes that genotype standard SNPs, we included probes that track presence or absence (and copy number) of selected regions of the mouse genome such as *Xce* and *Mx1*, and of sequences used in genetically modified mice such as Cre and Luciferase. Based on the results of over 4,000 samples genotyped, MegaMUGA provides a powerful tool for genetic analysis in complex populations including wild mice and a much needed platform for quality control of genetic stocks and mouse cell lines.

P-52: Generation of Pancreas CreER^{T2} Transgenic Mouse Lines for Time and Cell Specific Conditional Gene Inactivation

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Availability of conditional knock-out of most mouse genes from large-scale international mouse mutagenesis programs (IKMP and IMPC) is opening a new era for studying the mouse genome. In combination with promoter driven cre recombinases, this immense resource will not only allow to study the results of inactivation of any gene in the whole body but also to evaluate the function of these genes in a particular tissue or cell type. CreERT2 recombinases allow time controlled inactivation of a gene. Studying the effect of inactivation of genes at a specific age and studying genes resulting in lethal phenotypes is thus possible. The need of a large variety of cell specific deleter lines seems thus more and more essential.

At the ICS, we have generated about 50 Cre transgenic mouse lines expected to express the tamoxifen inducible CreERT2 recombinase in different target tissues and cells. These include different neuronal populations, immune system, adipose tissue, different cell populations in the digestive tract, pancreas, muscle, bone, immune system, reproductive tract, skin. For details, please see http://www.ics-mci.fr/mousecre/.

Here, we will present 3 fully characterized mouse lines for the study of metabolic diseases and phenotypes: Insulin1, Glucagon and Elastase-CreER^{T2}. For the Insulin1-CreERT2 line, the Cre expression is observed in the b-cells, the translocation in the nucleus is confirmed in the presence of Tamoxifen as expected for an inducible line. By breeding this line with Rosa26 reporter line, a specific LacZ staining is observed in the b-islet cells. This line was phenotyped (under chow diet) and no glucose intolerance was observed at the difference of the Rat Insulin Promoter (RIP)-Cre line. A comparative study (Ins1-CreERT2 versus RIP-Cre) was performed and will be detailed. The characterization of the Glucagon-CreERT2 (specific for alpha-cells) and elastase-CreERT2 lines (specific from acinar cells) will also be described.

P-53: The Y Chromosome Histone Demethylase KDM5D Modifies Recombination Hotspot Activity

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Mammalian meiotic recombination occurs at 1-2 kb long genomic sites, termed hotspots. The position and activity of hotspots is determined by the zinc finger protein PRDM9, which binds at hotspots and locally opens the chromatin by trimethylating histone 3 at lysine-4. Some hotspots are sex specific, behaving differently in female and male meiosis. To determine if these differences could be due to the presence of a male-specific regulator, we studied the role of *Kdm5d*, a histone 3 lysine-4 demethylase on the Y Chromosome and potential antagonist of *Prdm9*. We developed two new transgenic lines on C57BL/6J genetic background, one with a single copy of *Kdm5d* inserted in the X Chromosome, and another with two additional copies of *Kdm5d* on the Y Chromosome. We were unable to develop a line with autosomal insertion of *Kdm5d*. The transgenes were expressed in both female and male meiosis. The role of *Kdm5d* was examined by comparing recombination activity of male F1 hybrids of appropriate crosses between CAST/EiJ x C57BL/6J with three copies of *Kdm5d* on the Y Chromosome, F1 with two copies, one each on X and Y, and male F1 with one copy on Y, as well as the activity of F1 females with one copy on X and those with no copy. The effects of *Kdm5d* proved to be hotspot specific. Although there was no significant effect of transgenes on the regional (megabase scale) level of crossing over in either male or female transgenic crosses compared to control crosses, the activity of specific hotspots was significantly altered in all transgenic crosses, with some hotspots decreasing and others increasing in activity. We conclude that *Kdm5d* can modulate individual hotspot activity, but does not significantly change the sex-specific regional pattern of recombination along the centromere-telomere axis.

P-54: Voluntary Exercise Reduces AOM-Induced Intestinal Tumor Number in a Strain-Dependent Manner

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We tested the impact of exercise (voluntary wheel running) on latency and severity of intestinal tumor development induced by the chemical azoxymethane (AOM). Using 225 mice from standard inbred lines known to have high sensitivity to AOM (A/J, C57BL/6J, C58/J, I/LnJ), and emerging Collaborative Cross (preCC) lines known to have high levels of exercise, we tested exercise as a prevention behavior by providing access to running wheels during the 5 week AOM treatment period, after the 5 week AOM treatment period, or not at all. Tumor number and size were evaluated, and tissues collected for genome-wide transcriptional analysis. The impact of exercise during AOM exposure on tumor number, especially in the A/J and preCC 3140 strains, was striking. In both of these strains, exercise during AOM treatment led to a ~50% reduction in intestinal tumor numbers relative to no exercise. While exercise during AOM treatment also reduced tumor number in other strains, there were clear genotype x exercise interaction effects on AOM-induced tumor numbers. Exercise after AOM exposure had little impact, and exercise at any stage did not reduce tumor size. To evaluate mechanisms for the impact of exercise on tumor number, mRNA representing tumor and healthy colon tissue from A/J mice (the line with the greatest response to exercise in terms of tumor number reduction) is being evaluated for gene expression differences using the Affymetrix Mouse Gene 2.1 ST array. Additionally, new experiments are being conducted to further test the impact (and timing of) exercise on intestinal cancer in mice. Using treatment sizes of 24 A/J mice, we are testing the impacts of: a) 5 weeks in wheels before AOM treatment begins; b) 5 weeks in wheels during the AOM treatment; c) 10 weeks in wheels (5 weeks before AOM, 5 weeks during AOM); and d) no wheels.

P-55: Mapping Metabolic Traits in the Diversity Outbred Mouse Population

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Hyperlipidemia, a risk factor for cardiovascular disease, is a complex trait regulated by a variety of dietary and genetic risk factors. Inbred mice, which exhibit strain-specific variation in circulating lipoprotein levels, have effectively been used to model the genetic complexity of hyperlipidemia. 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 results into possible therapeutic targets. An alternative approach is the use of outbred strains for genetic mapping, such as the Diversity Outbred (DO) mouse panel recently developed by the Jackson Laboratory. Our current studies use the DO mice to map QTL for plasma lipid levels. DO mice were genotyped for 77,800 SNPs using the MegaMUGA array and founder haplotype states were predicted using a hidden Markov model. QTL mapping was carried out in R using a genetic model that incorporates reconstructed haplotypes and accounts for population structure. Our initial studies used 316 male and female DO mice fed a synthetic diet to map total cholesterol (TC) and triglyceride (TG) levels and identified a significant peak on Chromosome 1 (max LOD 8.02) and a suggestive peak on Chromosome 6 (max LOD 6.43) associated with TC. The Chromosome 1 QTL maps 1 Mb away from a well characterized gene regulating cholesterol (*Apoa2*). We identified two suggestive peaks on Chromosome 17 associated with TG. We have also measured plasma lipid levels and traits modeling atherosclerosis in an additional cohort of 300 female DO mice fed either a hyperlipidemia-inducing diet or a control diet; mapping results are pending. Our studies are intended to demonstrate the value of the DO resource to improve mapping resolution and aid in the identification of potential therapeutic targets for metabolic diseases.

P-56: Transgene Insertion Site Discovery by High-Throughput Sequencing of Mate Pair Libraries

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The first transgenic mice were produced by microinjection of DNA into pronuclei by Gordon and Ruddle in 1981, and since, transgenesis has become a reliable and powerful method for the creation of new mouse models. In mice, nearly 6,500 transgenic alleles (data from the Mouse Genome Database) have been created for a variety of purposes including tissue-specific expression of fluorescent proteins or other “reporters”, tissue-specific expression of recombinases (e.g. cre recombinase, which is widely used for conditional gene ablation), and for expression of human disease genes. In an effort to obtain a complete understanding of how insertion sites might contribute to phenotypic outcomes, to more cost effectively manage transgenic strains, and to fully understand mechanisms of instability in transgene expression, the Mutant Mouse Regional Resource Center at The Jackson Laboratory is exploring high-throughput sequencing based approaches to routinely identify transgene insertion sites. Similar to other molecular approaches to transgene insertion site discovery, high-throughput sequencing of standard paired-end libraries is hindered by low signal to noise ratios. Here, we describe a methodology and a novel algorithm for transgene insertion site discovery by high throughput sequencing of mate-pair libraries with insert sizes of 3-5 kb. We also show several examples of genomic regions that harbor transgenes, which have in common a preponderance of repetitive sequences and are gene poor.

P-57: Managing the Collaborative Cross: Effect of Housing Conditions on the Productivity of Large Breeding Programs

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The Collaborative Cross (CC) is a large panel of recombinant inbred mouse lines that is used to model the heterogeneous human population. The CC is an international project that aims to create and make widely available to the research community as many CC lines as possible. In the US, CC lines are made available through the UNC Systems Genetics Core Facility (SGCF) which currently has 48 lines that are deemed distributable (>90% homozygous). The size of the colony, the breeding schema, the cost and time are key elements to be considered for the efficient management of a mouse resource such as the CC. The two top priorities of SGCF at UNC are the maintenance of the distributable stocks and time and cost efficient access to CC mice by outside investigators. Our group has been collecting data on the management of the CC since it was established in 2005. This poster will review the impact of 1) relocation from facility to facility, 2) health status of the facility and 3) housing conditions (for example size of the cage and type of breeding) on the maintenance and productivity. We will use these data to justify our current breeding schema and the number of cages used to maintain the CC lines at the UNC SGCF.

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P-58: Genomic Shuffling and Recombination: Implications for Mammalian Chromosomal Evolution

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Analyzing how mammalian genomes are organized and by which mechanisms genome reshuffling is involved in speciation are fundamental for understanding the dynamics of genome evolution. It has been recently argued that chromosomal rearrangements could reduce gene flow and potentially contribute to speciation by the suppression of recombination. In this context, the aim of this study is to analyze how chromosomal rearrangements are organized and cross-related with recombination hotspots. Here we address these questions through a multidisciplinary approach, combining computational and experimental methods and by studying the genomes of pivotal mammalian species. We have first established whole-genome comparisons in order to detect homologous syntenic blocks (HSBs) and evolutionary breakpoint regions (EBRs) in both primates and rodent genomes. Subsequently, we analyzed the reorganized regions detected in relation to high-resolution genome-wide maps of recombination rates based on (i) SNPs data, and (ii) meiotic crossovers and double-strand breaks. By this way we provide a highly refined map of the reorganizations and evolutionary breakpoint regions in mammalian genomes. Our results reveal the existence of a relationship between chromosomal reorganizations and recombination. We detect that rearranged chromosomes presented significantly lower recombination rates than chromosomes that have been maintained collinear during evolution.

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P-59: A Systems Biology Approach to Identify Interactions Between Breast Cancer and Ageing

(See abstract SO-6 in the student/postdoc session)

P-60: Nuclease-Mediated Gene Knockout in an Allele Refractory to Gene Targeting in ES Cells

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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 for 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 and prepared for microinjection. 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 been reported to be effective for other genes. The microinjection of mRNA coding for TALENs also failed to generate mouse mutants, independent of the origin 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 that are undergoing genetic analysis. 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-61: A Comparative Phenotypic Analysis of Two C57BL/6N Substrains

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While the mouse inbred line C57BL/6J is widely used in mouse genetics, the International Knockout Mouse Consortium (IKMC) has established a mouse ES cell mutant resource employing C57BL/6N ES cells. A number of independent C57BL/6N subtrains being available from various animal providers, genetic differences accumulated over the years which likely may contribute to phenotypic variation. We analysed two C57BL/6N substrains (Tac and Crl) through a phenotyping pipeline developed by the International Mouse Phenotyping Consortium (IMPC),

namely the ImPRESS protocol including a sequence of tests that can be performed to fully characterize a mutant mouse. The colonies leading to the two substrains were separated 17 years ago, allowing genetic drift to take place.

In our design study the C57BL/6NTac substrain was bred internally and we compared them to C57BL/6NCrl either provided by the vendor at 6 weeks of age, or bred in house.

In summary, we were able to show that there were no significant phenotypic differences between both substrains, as long as both colonies are bred under the same husbandry conditions. In contrast, some phenotypic parameters were significantly different when C57BL/6NCrl mice are provided by the vendor at 6 weeks of age, in comparison to internal breeding.

In conclusion, with regard to the IKMC ES cell resources and mutants phenotyping studies, the two C57BL/6N substrains can be used for colony breeding, giving reliable phenotyping results as long as mutant and control mice are set up in the same environmental conditions.

P-62: Productivity in the Collaborative Cross: Effect of Genetic Diversity on Mating Success, Litter Size, and Sex Ratio

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The Collaborative Cross (CC) is a genetic reference population composed of more than 100 lines derived from 8 inbred laboratory founder strains. As the CC become increasingly inbred and reach the 90% homozygous threshold necessary to be considered Distributable, they are then available to any researcher through the UNC Systems Genetics Core Facility (SGCF). The SGCF currently has 53 CC lines listed as distributable. Once a CC line is listed, we start to collect data on its reproductive performance including litter size, number of productive matings, and sex ratio at weaning. Similar data has been collected for a subset of the 2,256 possible RIX (Recombinant Inbred intercrosses between CC lines) and for RIB (Recombinant Inbred Backcrosses between CC lines and another inbred line, typically a mouse model for a human disease) generated by mating CC to mice carrying FVB-Tg(C3-1-TAg)cJeg/Unc (C3Tag), a mouse cancer model. We also have preliminary data for four way intercrosses between different RIX lines. The litter and mating success have a wide range of variation within the CC, RIX, and RIB lines. Therefore, there are several CC and RIX lines with sex ratio distortion in favor of either females or males. Here we will present summary data for the reproductive parameter analysis in the distributable lines of the CC and discuss where genetic diversity may be responsible for the variations observed.

P-63: Discovery and Interpretation of Phenomic Data from the Harwell Aging Screen

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Understanding the risk factors which underlie the metabolic reactions, cell signaling and developmental pathways in human aging remain largely unknown. Discovery of candidate genes and phenotypes associated with aging mice will provide a wealth of information on age-related changes and gene molecular function. The Phenotype-driven screen after chemical mutagenesis of males with N-ethyl-N-nitrosourea (ENU) at MRC Harwell has been a productive method for identifying mouse models of aging.

Mutagenized mice (ranging from 30-100 per pedigree) are aged for 18 months with phenotypic assessments repeated at regular intervals (ranging from weekly to quarterly). To date >8000 G3 mice have completed the phenotypic pipeline with a further 7000 currently under assessment. Using robust statistical techniques for detecting phenodeviants we can determine different types of aging phenotypes from early to late onset and progressive, chronic or idiopathic phenotypes. Aging data can be found at mousebook.org.

Aging mice harboring interesting phenotypic changes are subsequently characterised by whole genome next generation sequencing (WGS). We have obtained all the possible induced single nucleotide variants (SNVs) in the G1 mouse and drilled down to the G3 for positive validation. This allows for the accurate estimation of protein coding and regulatory dark matter variants in the G1 mice. In addition we have predicted the occurrence of indels alongside the larger chromosomal aberrations.

Lastly our aim is to effectively integrate all our datasets to discover interesting and novel mouse models of human disease, here we present an overview of the data (genomic and phenotypic) and statistical methodology so far developed.

P-64: The FANTOM5 Project: Comprehensive Identification of Mammalian Promoters

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Using the Cap Analysis of Gene Expression (CAGE) and the single molecule sequencers, we have mapped transcription start sites (TSS) and systematically annotated across a broad panel of tissues, cell lines and primary cells in mouse and human. Thousands of deepCAGE libraries have been sequenced which produced almost comprehensive coverage of transcription starting sites (TSSs) for mouse and human, identifying more than180,000 reliable TSSs. The majority of the mammalian transcriptome has tissue/cell type influenced expression patterns indicating its role in multicellularity. We identified key known and novel transcription factor binding motifs within state-specific promoters. The mammalian gene expression atlas, constructed using the non-biased TSS frequency data, is the most comprehensive one to date. We generated a resource which identifies key transcription factors for specific cell types and confirm their relevance using publicly available phenotype information. We further functionally annotated uncharacterized genes by co-expression module clustering and a novel 'ranked sample ontology enrichment analysis' approach. The FANTOM5 TSS catalogue will be a reference transcriptome dataset widely used for the science community.

P-65: Novel QTLs Underlying Early-Onset, High-Frequency Hearing Loss in BALB/cA Mice

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Laboratory mouse strains offer important advantages as bioresources for hearing research. Many inbred mouse strains exhibit variable hearing capability and onset of age-related hearing loss (AHL). We found that common inbred BALB/cA mice express high-frequency specific hearing loss (HFHL) in tone-pip stimuli at 20 kHz and higher at 4 weeks of age compared with C57BL/6J mice, which are well-known models of AHL. In particular, the ABR thresholds at 32 and 36 kHz in the BALB/cA are about 20 dB SPL higher than those in C57BL/6J (B6) mice. In addition, the HFHL of BALB mice progressed to severe hearing loss by 12 weeks. We assessed in the F_1 crossed C57BL/6J and MSM/Ms with BALB/cA by analyzing ABR thresholds to 32 and 36 kHz stimuli. The ABR thresholds of (C57BL/6J x BALB/cA) F_1 and (MSM/Ms x BALB/cA) F_1 mice tested at 8 and 12 weeks of age were significantly different from those of BALB/cA mice. The ABR thresholds among the (BALB/cA x C57BL/6J) F_1 x BALB/cA backcrossed mice displayed a roughly bimodal rather than a bell-shaped normal distribution, suggesting a large contribution from 1 or a small number of loci. We identified quantitative trait loci on Chromosomes 8 and 19 that significantly affected hearing. Moreover, analysis of (BALB/cA x MSM/Ms) F_1 x BALB/cA backcrossed mice demonstrated that early-onset HFHL in BALB/cA is associated with *ahl* (753A) mutation of cadherin 23 gene. We conclude that quantitative trait loci on Chromosomes 8 and 19 are contributors to the HFHL of BALB/cA mice and that their effects depend on the $Cdh23^{ahl}$ genotype in this strain.

P-66: A Sensitized Whole Genome ENU Mutagenesis Screen Identifies a Novel Suppressor Region for Lethal Thrombosis in the Factor V Leiden Mouse

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

P-68: Comparison of Meiotic Recombination Rates Using Immunostaining and Chromosomal Painting

(See abstract SO-3 in the student/postdoc session)

P-69: Identification of Candidate Genetic Loci Responsible for a New Spontaneous-Microphthalmos Rat Strain, NAK/Nokh

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Microphthalmia, including anophthalmia and aphakia, is one of the most severe congenital eye disorders and is characterized by small or absent eyes or lens. We established an inbred anophthalmic rat strain derived from a Sprague-Dawley (SD) colony and designated it as nodai aphakia (*nak*). The NAK/Nokh rats had lower body size than wild-type rats and also did not have both eyes. During eye development in the NAK/Nokh rats, lens placodes and optic vesicles were observed at embryonic day (E) 12.5, and lens vesicles and the neural retina also developed

at E14.5. However, the development of these tissues was obviously delayed compared to that of wild-type and that is completely loss until E16.5. To identify the causative genetic loci, we generated 250 and 167 backcross progenies (N_2) between NAK/Nokh and Brown Norway (BN) or Wister (WI) rats, respectively. All the F_1 progenies showed normal eye weight (59.8 ± 2.23 mg), but various phenotypes such as bilateral or unilateral anophthalmia, microphthalmia (32.2 ± 8.9 mg), and normal eyes (54.0 ± 5.6 mg) were seen in both of N_2 progenies, with differing frequencies. The segregation ratio of affected and unaffected individuals was 179:71 and 75:92, respectively. The ratio of [(NAK/Nohk x BN) x NAK/Nokh] N_2 did not agree with the theoretical value for the autosomal recessive mode of inheritance. Thus, *nak* phenotypes might be modified by genetic background effects derived from different genomic components among strains. We found 2 candidate loci for *nak* at 13.6Mb intervals on Chromosome 16, with a highly significant LOD score of more than 8.0 obtained by genome-wide linkage analysis in 107 and 56 backcross progenies. Therefore, we speculate that the main factors responsible for the absent or small eyes phenotypes of *nak* should include the 2 mutations that we detected in distinct genes encoded in this genomic interval.

P-70: The Effects of Maternal Malnutrition in Utero on Behavioral Phenotypes of Mouse– Validation of DOHaD Theory in Mouse

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The developmental origins of health and disease paradigm (DOHaD) is a concept that fetal environmental factors affect the adult phenotypes. We are conducting experimental plans to validate the DOHaD theory in the developmental disorder with well-controlled experimental procedures using mouse. The in vitro fertilization (IVF) and embryo transfer (ET) technics were used for mouse production. ICR female mice were used as recipient and foster mother. The embryos were prepared from eggs and sperms of C57BL/6J to which normal diet was provided. The AIN93G as control diet, 5% low-protein diet (LP), and low-protein diet with supplemental folate (LP + FA) were provided as experimental diet to recipients since one month before ET to child birth. The body weight (BW) of mother and offspring, clinical biochemistry of mother, gene expression in the brain and the liver of neonates were examined. In order to examine the behavioral phenotypes of adolescent mice, we carried out following behavioral tests, open-field test; object exploration test; social-interaction tests; home-cage activity; light/dark-transition test; fear-conditioning test; tail suspension test. The maternal blood glucose and cholesterol was increased in LP group. The neonates of LP group exhibited lower BW relative to control group. The gene expression of neonatal brain obtained from LP group was slightly different from control group. In the adult offspring, gene-expression patterns in the brain were significantly changed in the LP and LP + FA group relative to AIN93G group. The offspring of LP group exhibited higher activity and lower attention to a novel object and a novel male relative to control group. Finally, we plan to examine methylation of genomic DNA, brain histopathology.

P-71: Advanced Humanized Rodent Models for ADMET Studies

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A crucial issue in preclinical drug development is the prediction of absorption, distribution, metabolism, excretion and toxicity (ADMET) profiles of drug candidates in humans. The accuracy of such predictions using commonly available preclinical animal models is often limited due to significant species differences in the pathways that define drug metabolism and disposition. The development of *in vivo* models which accurately reflect human biochemical pathways has been hindered by the limited number of genetic manipulations introduced in any given model described to date, restricting studies to few selected pathways of drug metabolism and disposition. To circumvent this problem, we have applied advanced genome targeting technologies to "humanize" mice for key components of human drug metabolism pathways, such as xenobiotic receptors, phase I/II enzymes, or drug transporter genes. In some cases, gene clusters spanning megabases have been manipulated to delete the mouse genes and replace them with the corresponding human counterparts. By using a combination of intercrossing and ES cell derivation and re-targeting, we have produced ADMET mouse models carrying multiple humanized loci in a single mouse line. As a result of this effort, we have now obtained a mouse line humanized in six different loci that to our knowledge represents the most advanced rodent model for ADMET studies generated so far.

P-72: *Dusp1* Knockout Mouse Response to Dietary Obesity

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We have localized a region on proximal mmu Chr 17 linked to sensitivity to dietary obesity in the mouse using a 129P2/Ola x C57BL/6N N2F1 intercross. A critical linkage region sub-localized by haplotype mapping contains a gene previously implicated in dietary obesity-*Dusp1*. Sequencing of *Dusp1* and surrounding regulatory regions revealed no SNPs or indels in the ORF or known gene regulatory domains between mapping strains. In order to examine a potential role for *Dusp1* in metabolic disease, we used a knockout mouse with a deleted critical exon,

Dusp1$^{tm1(KOMP)Wtsi}$, and compared wildtype (WT) C57BL/6NCrl and homozygous (HOM) mutant male (M) and female (F) mice (C57BL/6NCrl background) fed either a high-fat (HF) or a low-fat (LF) diet (Research Diets Inc., 45% and 10% fat calories respectively; n=6-11 mice per group). Both M and F HOM mice weighed less than WT controls on both diets after 12 weeks of feeding. HOM adiposity measured as absolute adipose depot weight, or as an index ([dissected adipose depot/lean weight] x 100) was less than WT. Insulin sensitivity assessed by an insulin tolerance test (1.0U/kg bw ip) was not different between HOM and WT for either sex or diet condition. And the area under the curve for an intraperitoneal glucose tolerance test (2g glucose/kg bw) was not different in LF or HF fed M and F HOM vs. WT. However, overnight fasting glucose was significantly reduced in HOM M HF mice (157mg/dl vs. 217mg/dl) and slightly reduced in F HF mice (131mg/dl vs 142 mg/dl). These data indicate that loss of *Dusp1* function reduces body adiposity but has a minimal effect on glucose homeostasis. Supported by NIH Grant DK085124.

P-73: The Sanger Mouse Genetics Project: High Throughput Large Scale Mutant Mouse Generation and Phenotyping

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Towards the International Mouse Phenotyping Consortium's aim of generating and characterising a knockout mouse for every protein coding gene, in the last year alone, the Sanger Mouse Genetics Project has generated a further 300 knockout mouse lines using the EUCOMM/KOMP targeted ES cell resource, bringing the total generated at Sanger to over 1000 (~5% of all protein coding genes). Phenotyping throughput has also increased with a further 187 lines completing the primary screen in the past 12 months, bringing the total to >650 lines complete. Data are openly available on the Sanger Mouse Portal (http://www.sanger.ac.uk/mouseportal/). An update of the project will be presented along with key trends in the data, such as genes causing recessive lethality are less likely to have a paralogue, and an example of how data from this primary screen can act as a springboard to stimulate specialist secondary characterisation.

Our screen has been extended through Wellcome Trust Strategic Award funding of consortia interested in three distinct biological areas: developmental biology, infection and immunity, and bone and cartilage disease. These initiatives will be described and pilot results presented.

P-74: Levels of the Mahogunin Ring Finger 1 E3 Ubiquitin Ligase Do Not Influence Prion Disease

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Prion diseases are rare, fatal neurodegenerative disorders associated with spongiform encephalopathy, a histopathology characterized by the presence of large, membrane-bound vacuolar structures in the neuropil of the brain. While the conversion of the normal form of prion protein (PrP) to a conformationally distinct pathogenic form is recognized to be the primary cause of prion disease, it is not clear how this conversion leads to spongiform change, neuronal dysfunction and death. Mice lacking the mahogunin, ring finger 1 (MGRN1) E3 ubiquitin ligase develop spongiform encephalopathy by 9 months of age but they do not accumulate the protease-resistant scrapie form of the prion protein or become sick. In cell culture, PrP aberrantly present in the cytosol was reported to interact with and sequester MGRN1. This caused endo-lysosomal trafficking defects similar to those observed when *Mgrn1* expression was knocked down, implicating disrupted MGRN1-dependent trafficking in the pathogenesis of prion disease. As these defects in cell culture were rescued by over-expression of MGRN1, we investigated whether manipulating *Mgrn1* levels influences the onset, progression or pathology of disease in mice inoculated with PrP^{Sc}. No differences were observed in mice lacking or over-expreressing MGRN1, indicating that disruption of MGRN1-dependent pathways does not play a significant role in the pathogenesis of transmissible spongiform encephalopathy.

P-75: A New Database Schema for High-Throughput Phenotype and Genotype Data in the Collaborative Cross

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The collaborative cross (CC) is an on-going, multi-institutional project, whose goal is to improve systems genetic methods using the mouse as a model organism. The CC resource is composed of hundreds of independently bred,

octo-parental recombinant inbred mouse lines. The System Immunogenetics (SI) website; systemsimmunogenetics.org, and its corresponding database were built to capture the genomic and phenotypic responses to pathogenic viral challenge (Influenza, SARS, West Nile Virus, and Ebola).

The SI website is built on the free, open source labkey software. The labkey platform provides performance, capacity and security framework necessary for managing this high throughput data.

Through a custom database schema and query/mining tools written into labkey, we can record the experimental design , track samples, mine genomic signatures, and ask biological questions across the massive amount of phenotypic, genomic, and hereditary information. Current phenotypic modules include Affymetrix Microarrays, Whole Body Plethysmography, Next Generation Sequencing, ELISA, Flow Cytometry, Histology, qPCR, T-Cell assays, and weight loss.

The SI website allows the user to query, mine and visualize large phenotypic data sets and monitor the progress of CC experiments. The user can track down the status of animals and search detailed information about the outcome of experiments through the use of controlled vocabulary. The user can also track data back to the recombinant inbred intercrosses (CC RIX lines) and lookup hereditary information. The user can visualize in real time the assignment of CC RIX lines to specific experiments. Since most of these tools are built within the web based interface no programming or data analysis experience is necessary to view results. The SI system is a collaborative web-based data management portal where different labs can share and exchange their data and results quickly. SI's development is on-going and will provide valuable insights into host genetics and pathogenesis on a variety CC projects.

P-76: Generation of a Knock-In Mouse Model Expressing a Regulatable TRP53 Protein Localized in the Mitochondria

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TP53 or components of its signaling pathways are inactivated during tumor formation or progression in the majority of human cancers. This suggests that TP53 acts as a block that has to be eliminated for tumor development. Consequently, restoring *TP53* function has long been considered an attractive anticancer therapeutic approach. To asses this possibility, we are using a mouse knock-in model that expresses TRP53ERTAM protein, whose function can be reversibly switched on and off in vitro and in vivo. Using this model we have been able to show that transient restoration of TRP53 in a mouse model of chronic myeloid leukemia delays disease onset and prolongs the life of leukemic mice[1].

In recent years, it has been shown that TP53 can induce apoptosis and necrosis acting directly on the mitochondria, independently of its function as a transcription factor. To explore the possible anti tumor activity of mitochondrial TRP53 we have generated a novel knock-in mouse model that expresses a regulatable TRP53 protein fused to a canonical mitochondrial localization signal. This protein, TRP53mitoERTAM, is inactive in the absence of tamoxifen but can be switched to a functional status in the presence of the drug. As expected, in the absence of tamoxifen, these mice behave as *Trp53* null mice developing early onset tumors. We will use this model to analyze in vivo the role of mitochondrial TRP53 in tumor suppression and other pathophysiological processes such as ischemic necrosis, and eventually to identify novel therapeutic targets. We will present a progress report of our research using this mouse model.

1. Velasco-Hernández, T; Vicente-Dueñas, C; Sánchez-García, I; Martín-Zanca, D. *p53* restoration kills primitive leukemia cells *in vivo* and increases survival of leukemic mice. *Cell Cycle* 12, 122-133, 2013.

P-77: An Integrative Meta-Analysis Method to Reveal Age-Related Cross-Tissue Pathways

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Aging is a major biological process and a risk factor for many diseases, characterized by a progressive decline in organ functionality as age increases. This highly complex phenomenon has been substantially re-evaluated recently through large scale Systems Biology approaches, which in turn contextualize the aging effect through changes on genome scale networks. Towards this orientation, we unravel the cross-tissue age-associated gene interactome changes through an "ensemble" network inference methodology, driven by the fact that the "wisdom of crowd" among the results of various network inference algorithms leads to more reliable and robust networks.

Based on large cohort of C57BL/6 mouse microarray data from 16 tissues of the AGEMAP study, we isolated the youngest (1 month) and oldest samples (24 months) and discarded the middle-aged ones. On second level, an ensemble of gene regulatory networks reconstruction methods was employed to infer a reliable network on global scale. The ensemble included Aracne, CLR, MRNET, PCIT, GENIE3 algorithms and the Borda voting counting scheme was used to infer the consensus network.

With the scope to reveal the transcriptional changes during age, we performed difference analysis on the consensus network of each tissue. In detail, we assigned weights onto the network so that the weight represents the change in mutual information between old and young mice for the corresponding gene pair. Using again a Borda voting counting scheme, we defined the cross-tissue consensus difference network based on the 16 tissue difference networks. Finally, we applied a module-detecting algorithm to identify subgraphs that significantly loosen or strengthen with age.

The proposed framework is a robust and highly confident approach for designating the pathways significantly declining with age. Our work is applicable in other complex diseases or biological processes by shifting the interest from the changes on individual genes towards the changes in gene relations.
