IMGC 2018

32nd International Mammalian Genome Conference

11-14 November 2018

Wyndham Grand Rio Mar

Rio Grande, Puerto Rico

Welcome to the 32nd International Mammalian Genome Conference

Wyndham Grand Rio Mar
Rio Grande, Puerto Rico
November 11-14, 2018

We welcome you to the 32nd Annual Conference of the International Mammalian Genome Society and to the Wyndham Grand Rio Mar, Rio Grande, Puerto Rico. We are planning an exciting meeting with emphasis on cutting-edge research in the fields of mammalian genetics and genomics, with experts in a wide range of biology specialties participating.

The meeting will begin with five Bioinformatics Workshops followed by a Student Satellite Symposium that offers the opportunity for budding scientists to compete for awards and presentation slots in the main meeting. The conference will once again include the popular mentoring lunch where those looking for career advice can interact with established scientists.

The main conference will feature sessions on:

- Technical Advances and Resources
- Translational & Systems Genetics
- Development, Epigenetics and Stem cells
- Human disease models—cancer and environmental factors; infection and immunology; metabolic; neurobehavioral
- Comparative Genomics, Computational Methods & Evolution

***Puerto Rico** will captivate you with dynamic culture, rich heritage, local flavors, stunning beaches, and amazing adventures. Whether you're passionate about nature and love to explore, or you want to experience urban settings full of culture, activities and nightlife – there is something for you on this island. Enjoy the beach at the Wyndham Grand Rio Mar. Explore the Rainforest El Yunque or fall in love with Old San Juan as you become immersed in tropical colors, colonial architecture, epic fortresses, boutique and high-end shopping, and cultural events for the whole family. Puerto Rico is home to 3 of the 5 bioluminescent bays in the world! The enchantment of Puerto Rico will also be found in the hospitality of its people.*

We hope to give you opportunities to experience great scientific interactions as well as some of the wonder of this island.

We welcome you to Puerto Rico and the 32nd IMGC!

The 32nd International Mammalian Genome Conference

Rio Mar, Puerto Rico
November 11-14, 2018

Sunday, November 11th		
9:00 AM - 12:00 PM	Workshops	
12:00 PM - 1:00 PM	Lunch (not provided)	
1:00 PM - 5:30 PM	Trainee Symposium	Rio Mar 5
3:00 PM - 3:30 PM	Break	
6:00 PM- 8:00 PM	Dinner	Marbella Gardens
8:00 PM	Official Opening of IMGC 2018	Rio Mar 5
8:15 PM	Verne Chapman Memorial Lecture	Rio Mar 5

Monday, November 12th		
6:30 AM - 8:15 AM	Breakfast	Caribbean Ballroom 2-3
8:15 AM	Opening remarks	
8:30 AM - 10:00 AM	Session 1	Rio Mar 5
10:00 AM - 10:20 AM	Break	
10:20 AM - 11:50 AM	Session 2	Rio Mar 5
11:50 AM - 2:30 PM	Lunch and Posters (Odd)	Caribbean Ballroom 2-3/Rio Mar 6-10
11:50 AM - 2:30 PM	Nomenclature Lunch	El Morro 1-2
2:30 PM - 4:30 PM	Session 3	Rio Mar 5
5:15 PM	Buses leave for BLB or OSJ	El Yunque Foyer

Tuesday, November 13th		
6:30 AM - 8:30 AM	Breakfast	Caribbean Ballroom 2-3
8:30 AM - 10:05 AM	Session 4	Rio Mar 5
10:05 AM - 10:25 AM	Break	
10:25 AM -11:55 AM	Session 5	Rio Mar 5
11:55 AM - 1:30 PM	Lunch / Mentor Lunch	Caribbean Ballroom 2-3
1:30 PM - 3:00 PM	Forward Genetics Panel (Hosted by INFRAFRONTIER)	Rio Mar 5
3:00 PM - 4:00 PM	IMGS Business Meeting	Rio Mar 5
4:00 PM- 6:00 PM	Posters (Even) with rum tasting	Rio Mar 6-10
6:00 PM - 7:00 PM	One hour reception / bar	Ocean Terrace
7:00 PM - 8:00 PM	Dinner on your own	
8:00 PM - 9:30 PM	Session 7	Rio Mar 5

Wednesday, November 14th		
6:30 AM - 8:30 AM	Breakfast	Caribbean Ballroom 2-3
8:30 AM - 10:00 AM	Session 8	Rio Mar 5
10:00 AM - 10:20 AM	Break	
10:20 AM - 11:50 AM	Session 9	Rio Mar 5
11:50 AM - 1:30 PM	Lunch	Caribbean Ballroom 2-3
11:50 AM - 1:30 PM	Secretariat lunch	El Morro 1-2
1:30 PM - 2:45 PM	Session 10	Rio Mar 5
2:45 PM- 3:05 PM	Break	
3:05 PM - 4:20 PM	Session 11	Rio Mar 5
4:20 PM	Close of sessions	
5:15 PM	Buses leave for Hacienda Siesta Alegre	El Yunque Foyer

GENERAL INFORMATION

Certificate of Attendance

Certificates will be emailed to delegates after the conference upon request.

Insurance

The Conference Organizers cannot accept any liability for personal injuries or for loss or damage to property belonging to delegates, either during, or as a result of the meeting. Please check the validity of your own personal insurance before traveling.

Meeting Etiquette

Delegates are advised that they are not allowed to take photographs of any posters or presentations without the author's/presenter's consent. Delegate should also obtain consent from an author before citing any of their work that was presented at the conference.
Cell phones or other devices that have notification sounds should be switched off or placed on silent during sessions. Please also respect speakers and other delegates and refrain from talking during presentations. Thank you for your cooperation.

Social Media Policy

Live tweeting of presentations is allowed unless the speaker explicitly opts out by stating so at the start of their talk. Attendees are encouraged to post their thoughts on exciting scientific advances and other meeting events. Use #IMGC2018 to let everyone know what is happening at the meeting.

Internet Access

Internet access at the Wyndham Grand Rio Mar should be freely available to you both in the meeting space and the hotel rooms. If a code is needed, it will be provided at the conference.

Catering

The opening reception and dinner (ticketed) will be in the beautiful Marbella Gardens (weather permitting). Breakfast (Monday through Wednesday) will be by ticket (or cash for guests) in the Marbella Restaurant. Lunches will be in the Caribbean Ballroom 2-3 near the general session room. The final Awards Dinner (ticketed) will be at the Hacienda Siesta Alegre. Transportation by bus to this stunning location will be provided. Note that the opening and closing dinners are scheduled to be outdoors on grassy areas, so plan footwear accordingly.
If you requested a special diet at the time of registration, you should have a colored dot on your nametag to notify the serving staff, but feel free to mention your need to the serving staff as needed. Since most meals will be served buffet style, you should be able to identify any special dietary requirements.

Exhibition and Poster Times

The Exhibition area will be open during all conference hours and your poster can be displayed starting at noon on Sunday, November 11 and should be removed by 3 PM on Wednesday, November 14.
Presenters should be by their posters at the following times:

Odd Numbered Posters	Monday, November 12 from after eating lunch until 2:30 PM
Even Numbered Posters	Tuesday, November 13 from 4 until 6 PM

Registration Desk

All delegates will receive their name badge, meeting documents, and meal tickets upon arrival at the IMGC registration desk which will be located in the hotel lobby on Sunday and near the general session room beginning on Monday. The registration desk will be open during all normal conference hours starting at 8 AM on Sunday, November 11.

Speaker Presentation Check-in

Presenters should bring their presentation on a USB drive to the conference. All presentations will be loaded onto a main presentation computer in the session room. Presenters will not be using their own laptops for presentations. Please check your presentations in at least one session prior to your presentation time.

Mentoring Lunch
Those students and post docs interested (which we hope is all of you!) in participating in the Mentoring Lunch on TUESDAY, November 13, should look for the designated tables in the Caribbean Ballroom 2-3 when entering for that meal.

Optional Events
Monday, November 12
1) There will be **bus transportation from the Wyndham Grand Rio Mar to Old San Juan** departing at 5:15 PM. (Ticketed)
The buses will depart from Old San Juan at approximately 9:45 PM. You are welcome to arrange your own transportation back to the Wyndham from Old San Juan (Uber, taxi, etc.) Therefore, the buses from Old San Juan will NOT be checking to see if all are on board before departure. Please confirm with driver pick up location and time.
OR
2) **Bioluminescent Bay Tour** (ticketed event). Buses will depart the Wyndham Grand Rio Mar at 5:15 PM. It is suggested that you wear swimwear under shorts, etc. and water shoes or flip flops as you are very likely to get wet. You may also want to take a towel and a change of clothing for the ride home. If you ordered a box meal, it will be given to you either as you board or on the bus. The tour is in two-person kayaks and should be 90-120 minutes on the water. Buses will return you to the Wyndham after the tour.

Tuesday, November 13
4-6 PM Rum tasting during the poster session in Caribbean 6-10 by Nativo Rumeliers.
6-7 PM Hors d'oeuvres and cocktails will be served on the Ocean Terrace.
These are non-ticketed events.

Enjoy the 32nd IMGC!!

IMGS Secretariat

Martin Hrabe de Angelis, President (2020)
Linda Siracusa, Vice-President (2022)
Teresa Gunn, Past President (2018)

Thomas Keane (2018)
Lluis Montoliu (2018)
Bill Pavan (2018)
Jake Moskowitz (VM Chapman Award Winner)

Kuniya Abe (2019)
Lauren Tracey (VM Chapman Award Winner)

Ruth Arkell (2020)
Laura Reinholdt (2020)

IMGS Nomination and Election Committee

James Amos-Landgraf (2018)
David Aylor (Chair--2018)
Elena de la Casa Esperon (2018)

Marty Ferris (2019)
Viive Howell (2019)

32nd IMGC Scientific Organizers

Fernando Pardo-Manuel de Villena
David Threadgill
Paola Giusti-Rodriguez

IMGS COO

Darla Miller

International Committee on Standardized Genetic Nomenclature for Mice

Ruth Arkell	Doug Marchuk	David Threadgill
Judith Blake	Monica McAndrews	Jacqui White
Elspeth Bruford	Lluis Montoliu	Laurens Wilming
Teresa Gunn	Valerie Schneider	
Ann-Marie Mallon	Cynthia Smith (Chair)	

EXHIBITORS/ SPONSORS

The International Mammalian Genome Society would like to thank the following exhibitors and sponsors for their support:

DZD
Deutsches Zentrum
für Diabetesforschung

GENE EDIT BIOLAB

INFRAFRONTIER
mouse disease models

AWARDS

The Secretariat and members of The International Mammalian Genome Society are proud of the students, postdoctoral fellows and junior faculty who present oral and poster presentations at the annual meeting. The outstanding contributions of these young scientists are recognized through several awards.

The first of these awards is the Verne Chapman Young Scientist Award, which is given to the most outstanding oral presentation from a postdoctoral fellow or student. This is a monetary award of $500 and a two-year position on the IMGS secretariat that reflects Dr. Chapman's dedication to mentoring junior scientists.

A selection of publishing companies have sponsored this year's presentation prizes, which are a one year subscription to their journal or a donation of books. A cash award is given by The Genetics Society of America.

These awards are given to the most outstanding poster or oral presentations by graduate students and postdoctoral fellows, and are chosen by members of the IMGS Secretariat and other judges during the course of the meeting.

This year the sponsors are

STUDENT SCHOLARSHIPS

Funding for student scholarships was made possible by from NHGRI and NIEHS under grant R13HG002394 and from the Deutsches Zentrum für Diabetesforschung.

E-mail: service@geneeditbiolab.com

CRISPR & Gene Targeting Experts
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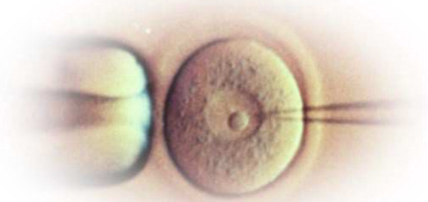
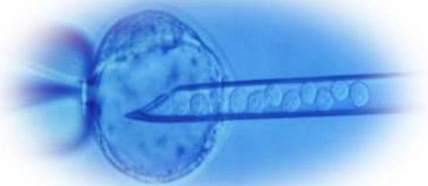
Gene Edit Biolab (GEB), located in Atlanta, GA, is specialized in generating customized genetically modified mouse & cell line models. Our expertise helped numerous investigators around the nation including *Baylor College of Medicine, John Hopkins University, University of Virginia, Emory University, Tulane University, Vanderbilt University*, etc.



Services:

- CRISPR/Cas9-based gene editing in mouse or cell line
  - ◊ Constitutive & conditional knockout
  - ◊ Constitutive & conditional knock-in (point mutation, tag, gene replacement)
- ES cell gene targeted mouse
  - ◊ From construct design to germline F1 or F2
- Blastocyst injection from consortium ES cells (KOMP, EUCOMM etc.)
- Transgenic mouse (plasmid or BAC)
- Humanized animal models (complete cDNA or BAC)



GEB Advantage:

- Proven success record, 99% project success rate
- Full guarantee & fast turnaround in as little as 6 months (from design to F1)
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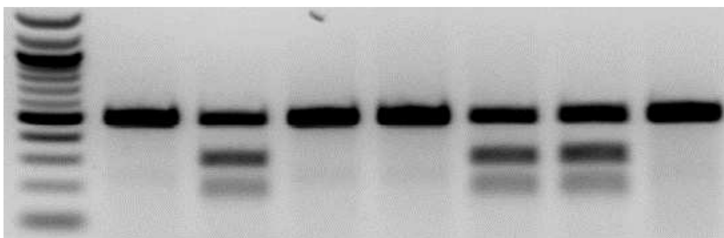

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The 32nd International Mammalian Genome Conference

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November 11-14, 2018

Saturday, November 10th		
2:00 PM - 6:00 PM	Secretariat Meeting	Caribbean Salon 3

Sunday, November 11th		
9:00 AM - 12:00 PM	Workshops	
	CRISPR	Egret
	Ensembl	Heron
	GeneWeaver	Board
	IMPC	Morro 1
	Next Gen	Morro 2
12:00 PM - 1:00 PM	Lunch (not provided)	
1:00 PM - 5:30 PM	Trainee Symposium Chairs: Teresa Gunn / Linda Siracusa	Rio Mar 5
1:00 PM	TS-1: Whole Genome Sequence Analysis Issues Revealed by Evaluation of a Linkage Peak in a Dominant ENU Suppressor Mouse Line	Marisa A Brake
1:15 PM	TS-2: Identification and Characterization of Transposable Elements in Genetic Reference Populations	Anwica Kashfeen
1:30 PM	TS-3: A mouse translocation dysregulating topologically – associating *Galnt17* and *Auts2* genes associated with behavioral and neuropathological phenotypes related to the human AUTS2 syndrome	Chih-Ying Chen
1:45 PM	TS-4: Genomic signatures of age-dependent hybrid male sterility in the mouse	Samuel Widmayer
2:00 PM	TS-5: Investigating genetic diversity of centromeres: a repetitive, non-coding, unassembled region of the genome	Uma Arora
2:15 PM	TS-6: Cellular systems genetics implicates KRAB zinc finger proteins in mediating variation in chromatin state in pluripotent stem cells	Candice Byers
2:30 PM	TS-7: Inter-individual variability in epigenetic and genotoxic responses to 1,3-butadiene in a population-based Collaborative Cross mouse model	Lauren Lewis
2:45 PM	TS-8: Evaluating the transcriptomic plasticity of stem cell quiescence depth with age	Joseph Palmer
3:00 PM - 3:30 PM	Break	
3:30 PM	TS-9: Discovering Genetic Modifiers in a new Mouse Model of Niemann-Pick Disease, Type C	Jorge L Rodriguez-Gil
3:45 PM	TS-10: The Loss-of-Function Intolerant *Actr2* Gene is Resistant to Gene Targeting and Genome Editing	Amy E Siebert
4:00 PM	TS-11: Alterations in metabolism and circadian rhythm in a *Pax6* mutant mouse line	Nirav Florian Chhabra
4:15 PM	TS-12: Pairing systems genetics and longitudinal analyses to identify regulators of complex lung disease phenotypes	Lauren Donoghue
4:30 PM	TS-13: Variable effects of steroid treatment on patient derived keloid fibroblasts – is underlying genetic/epigenetic heterogeneity the cause?	Yuna Son

4:45 PM	TS-14: *Msr1* modulates infarct volume through a collateral vessel-independent mechanism	Leandro Prado
5:00 PM	TS-15: Dissecting the role of TLR4 signaling during SARS- and SARS-like CoV infection in mice using the Collaborative Cross reference population and multiple mapping approaches	Sarah R Leist
5:15 PM	TS-16: Progression of colorectal cancer through epidermal growth factor receptor (EGFR)-independent mechanisms	Jorge Jaimes-Alvarado
6:00 PM- 8:00 PM	Dinner	Marbella Gardens
8:00 PM	Official Opening of IMGC 2018 Chair: Martin Hrabě de Angelis	Rio Mar 5
8:15 PM	Verne Chapman Memorial Lecture O-1 : Genome-Wide Dynamics of Chromatin Modifiers	Terry Magnuson

Monday, November 12th		
6:30 AM - 8:15 AM	Breakfast	Caribbean Ballroom 2-3
8:15 AM	Opening remarks	
8:30 AM - 10:00 AM	Session 1 Chairs: Fernando Pardo-Manuel de Villena / Kärt Tomberg	Rio Mar 5
8:30 AM	O-2: NAD Deficiency, Congenital Malformations and Vitamin B3 Supplementation	Sally Dunwoodie
9:00 AM	O-3: Comparative genetics and genomics of mouse strains and species at Mouse Genome Informatics (MGI)	Carol J Bult
9:15 AM	O-4: Accounting for haplotype uncertainty in QTL mapping of multiparental populations using multiple imputation	Greg Keele
9:30 AM	O-5: The dynamic transcriptional landscape of mammalian organogenesis at single cell resolution	Junyue Cao
9:45 AM	O-6: Exploring rodent genetic and phenotypic diversity for biomedical and environmental applications	Thomas M Keane
10:00 AM - 10:20 AM	Break	
10:20 AM - 11:50 AM	Session 2 Chairs: Thomas Keane / Sarah Leist	Rio Mar 5
10:20 AM	Trainee	
10:35 AM	Trainee	
10:50 AM	O-7: Targeted investigation of PhyloCSF regions identifies more than 100 novel protein-coding genes on the mouse reference genome	Jane Loveland
11:05 AM	O-8: Background-dependent mutation rate and strong deleterious effects of *de novo* transposition of endogenous retrovirus in mouse inbred strains	Fernando Pardo-Manuel de Villena
11:20 AM	O-9: Genetic variation influences ground state pluripotency in mouse embryonic stem cells through a hierarchy of molecular phenotypes	Daniel Skelly
11:35 AM	O-10: Updating The Mouse Genome: The Impact of Mutation and Drift on C57BL/6J	Gary A Churchill
11:50 AM - 2:30 PM	Lunch and Posters (Odd)	Caribbean Ballroom 2-3/Rio Mar 6-10
11:50 AM - 2:30 PM	Nomenclature Lunch	El Morro 1-2
2:30 PM - 4:30 PM	Session 3 Chairs: Bev Mock / Danny Arends	Rio Mar 5
2:30 PM	O-11: Animal Modeling in the Era of Precision Medicine	Elizabeth C Bryda
3:00 PM	O-12: Identification of meiotic non-crossovers in mice	Vaclav Gergelits
3:15 PM	O-13: The biological importance of the unique DNA binding properties of MECP2	Rebekah Tillotson
3:30 PM	Trainee	

Time	Event	Speaker / Location
3:45 PM	O-14: Intestinal Tumors in Hybrids from Crosses of Collaborative Cross Mice with a Sensitized Strain Reveal an Array of Phenotypes	Elena Mogilyansky
4:00 PM	O-15: PRDM9 functions as a specialized meiotic pioneer factor that activates recombination hotspots by recruiting a nucleosome remodeling factor and opening chromatin	Christopher Baker
4:15 PM	O-16: Scarless repair of kidney injury in African Spiny mice (*Acomys cahirinus*)	David Beier
5:15 PM	Buses leave for BLB or OSJ	El Yunque Foyer

Tuesday, November 13th

Time	Event	Speaker / Location
6:30 AM - 8:30 AM	Breakfast	Caribbean Ballroom 2-3
8:30 AM - 10:05 AM	Session 4 Chairs: Kuniya Abe / Lisa Gralinski	Rio Mar 5
8:30 AM	Mary Lyon Awardee Lecture O-17: Evolution and function of the mammalian pseudoautosomal region	Beth L Dumont
8:50 AM	Trainee	
9:05 AM	Trainee	
9:20 AM	O-18: Dissecting the mechanistic basis of the Dobzhansky–Muller incompatibility causing F1 hybrid sterility in *Mus musculus*	Tanmoy Bhattacharyya
9:35 AM	O-19: Gene x environment interactions cause sperm decline and male infertility in mice	David Aylor
9:50 AM	O-20: Deficient LRRC8A-dependent Volume-Regulated Anion Channel (VRAC) activity is associated with male infertility in mice	Fernando Benavides
10:05 AM - 10:25 AM	Break	
10:25 AM -11:55 AM	Session 5 Chairs: Kent Hunter / Rebekah Tillotson	Rio Mar 5
10:25 AM	O-21: Targeting the MYC G-Quadruplex Induces Cancer Cell Death	Beverly Mock
10:40 AM	O-22: The pluripotency regulator *Prdm14* hijacks hematopoietic regulatory *Cbfa2t3* to initiate leukemia in mice	Lauren Tracey
10:55 AM	O-23: The use of an untargeted metabolomics approach to determine the functional consequences of differences in host genetics and the gut microbiota in Apc^{Min}/+ intestinal tumorigenesis	Jacob Moskowitz
11:10 AM	O-24: Dynamic chromatin underlies intestinal stem cell differentiation	Jesse Raab
11:25 AM	O-25: Determining the mechanism for lung lipid defects in a *Mecp2* mutant mouse model for Rett syndrome	Neeti Vashi
11:40 AM	O-26: Standardized characterization of mouse models for genetic diseases with intellectual disabilities unravelled unique behavioural profiles	Yann Herault
11:55 AM - 1:30 PM	Lunch / Mentor Lunch	Caribbean Ballroom 2-3
1:30 PM - 3:00 PM	Forward Genetics Panel (Hosted by INFRAFRONTIER) Chair: Martin Hrabě de Angelis	Rio Mar 5
1:30 PM	O-27: Mutagenesis with real-time assignment of cause and effect	Bruce Beutler
2:00 PM	Panel Discussion	
3:00 PM - 4:00 PM	IMGS Business Meeting	Rio Mar 5
4:00 PM- 6:00 PM	Posters (Even) with rum tasting	Rio Mar 6-10
6:00 PM - 7:00 PM	One hour reception / bar	Ocean Terrace
7:00 PM - 8:00 PM	Dinner on your own	
8:00 PM - 9:30 PM	Session 7 Chairs: David Threadgill / Amy Siebert-McKenzie	Rio Mar 5

8:00 PM	O-28: Role for genetic variation in the NLRP1 inflammasome locus in Influenza A Virus pathogenesis	Kelsey E Noll
8:15 PM	O-29: Mapping of susceptibility alleles for Ebola hemorrhagic fever pathogenesis and resistance	Alexandra Schäfer
8:30 PM	O-30: Interactions between the host genome and the gut microbiome determine susceptibility to CNS autoimmune disease	Dimitry Krementsov
8:45 PM	O-31: Using a reduced complexity cross to identify host susceptibility factors to SARS-CoV infection	Lisa E Gralinski
9:00 PM	O-32: Of mice and men – the host response to influenza virus infection	Klaus Schughart
9:15 PM	O-33: Genetic Regulation of Airway Mucins and Consequences on Host Defense in Mice	Samir Kelada

Wednesday, November 14th

6:30 AM - 8:30 AM	Breakfast	Caribbean Ballroom 2-3
8:30 AM - 10:00 AM	Session 8 Chairs: Monica Justice / Nirav Chhabra	Rio Mar 5
8:30 AM	O-34: Epigenetic control of complex disease susceptibility	J Andrew Pospisilik
9:00 AM	O-35: Using Advanced Mouse Populations to Probe Genes and Networks that Drive Addiction-Related Behavior	Lisa Tarantino
9:15 AM	O-36: Exploiting dynamic open chromatin in mouse dopamine neurons reveals Parkinson-associated variation in an *SNCA* enhancer: a paradigm for illuminating functional noncoding variation	Sarah McClymont
9:30 AM	O-37: Genetic control of physiological response to carbohydrate consumption	Anna Salvador
9:45 AM	O-38: Assessment of early-life lead exposure in a genetically heterogeneous mouse population	Danila Cuomo
10:00 AM - 10:20 AM	Break	
10:20 AM - 11:50 AM	Session 9 Chairs: Laura Reinholdt / Leandro Prado	Rio Mar 5
10:20 AM	O-39: Generation and validation of increasingly complex alleles by genome editing	Lydia Teboul
10:35 AM	O-40: Completion of first pass GENCODE gene annotation for the mouse reference genome	Adam Frankish
10:50 AM	O-41: Elucidating the putative regulatory role of GWAS-associated neural disease variants using STARR-Seq	William Law
11:05 AM	O-42: Genetics of seizure sensitivity in the Collaborative Cross	John R Shorter
11:20 AM	O-43: Exploring high-throughput sequencing data using Multi-string Burrows Wheeler Transforms	Leonard McMillan
11:35 AM	O-44: The expanded BXD family : A cohort for experimental systems genetics and personalized precision medicine	Danny Arends
11:50 AM - 1:30 PM	Lunch	Caribbean Ballroom 2-3
11:50 AM - 1:30 PM	Secretariat lunch	El Morro 1-2
1:30 PM - 2:45 PM	Session 10 Chairs: Bill Pavan / Paola Giusti-Rodriguez	Rio Mar 5
1:30 PM	O-45: A suppressor screen in *Mecp2* mice implicates the DNA damage response in Rett Syndrome pathology	Monica J Justice
1:45 PM	O-46: *Uqcrh* KO mouse mimics mitochondrial respiratory chain disorder	Valérie Gailus-Durner
2:00 PM	O-47: Discovery of new genes related to human metabolic disorders by high throughput mouse phenotyping	Martin Hrabě de Angelis

2:15 PM	O-48: RGD's updated and redesigned Phenotypes and Models Portal: Data for precision models of human disease	Jennifer R Smith
2:30 PM	O-49: Antipsychotic behavioral phenotypes of Collaborative Cross recombinant inbred inter-crosses (RIX)	Paola Giusti-Rodriguez
2:45 PM- 3:05 PM	Break	
3:05 PM - 4:20 PM	Session 11 Chairs: Ruth Arkell / John Shorter	Rio Mar 5
3:05 PM	O-50: Many shades of gray: a comparative analysis of pheomelanin-specific mouse mutants	Dawn E Watkins-Chow
3:20 PM	O-51: Utility of the Pirc rat model of Familial Adenomatous Polyposis as a platform to test the role of the gut bacteria *Desulfovibrio vulgaris* on adenoma development	James Amos-Landgraf
3:35 PM	O-52: Update on housing density studies using research mice: time for a change?	Karen L Svenson
3:50 PM	O-53: Misregulation of an activity-dependent splicing network impacts neuronal translation and underlies autism spectrum disorders	S P Cordes
4:05 PM	O-54: Machine learning predicts association between *Ythdf3* and cognition in a mouse model of Alzheimer's disease	Anna L Tyler
4:20 PM	Close of sessions	
5:15 PM	Buses leave for Hacienda Siesta Alegre	El Yunque Foyer

Posters (Odd)

P-1 *INFRAFRONTIER – resources and services to advance the understanding of human health and disease using mammalian models,* Asrar Ali Khan

P-5 *Origin and Fate of* de novo *Insertions of Transposable Elements in the Collaborative Cross,* Timothy A Bell

P-7 *RNA-Seq Technologies Identify Causal Mutations in Mice with Mendelian Disorders,* David Bergstrom

P-9 *Content and performance of MiniMUGA, a new tool to improve rigor and reproducibility in mouse research,* Matthew W Blanchard

P-11 *Whole Genome Sequence Analysis Issues Revealed by Evaluation of a Linkage Peak in a Dominant ENU Suppressor Mouse Line,* Marisa A Brake

P-13 *Cellular systems genetics implicates KRAB zinc finger proteins in mediating variation in chromatin state in pluripotent stem cells,* Candice Byers

P-15 *Alterations in metabolism and circadian rhythm in a* Pax6 *mutant mouse line,* Nirav Florian Chhabra

P-17 *Genetic modification in the rat model: Streamlining new genome editing techniques,* Daniel Davis

P-19 *Zebrafish Crispr/Cas9 screen for cross-species functional conservation of genes regulating mouse pheomelanin synthesis,* Colten D Eberhard

P-21 *The German Mouse Clinic: a collaboration-based platform for phenotype characterization,* Helmut Fuchs

P-23 *Genome sequencing of PL/J, QSi3, and QSi5 identifies novel coding and truncating variants for divergent litter rearing capacity,* Darryl Hadsell

P-25 *Transcriptional Profiling of a Pre-Symptomatic Rett Syndrome Mouse Model,* Laura M Hergott

P-27 *Progression of colorectal cancer through epidermal growth factor receptor (EGFR)-independent mechanisms,* Jorge Jaimes-Alvarado

P-29 *Identification and Characterization of Transposable Elements in Genetic Reference Populations,* Anwica Kashfeen

P-31 *Rigorously Free: Mutant Mouse Models at the NIH MMRRC,* Chris Lossin

P-33 *Dissecting the role of TLR4 signaling during SARS- and SARS-like CoV infection in mice using the Collaborative Cross reference population and multiple mapping approaches,* Sarah R Leist

P-35 *Deletion of mouse* Fmr1nb *gene leads to errors in chromosome alignment and reduced male fertility,* Diana Lustyk

P-37 *EvalDNA: A machine-learning based tool for the comprehensive evaluation of mammalian genome assembly quality,* Madolyn MacDonald

P-39 *One-step generation of conditional knockout mice by CLICK,* Tomoji Mashimo

P-41 *Representation of Orthology in the Alliance of Genome Resources: The Mouse Perspective,* Monica S McAndrews

P-43 *Evaluating the transcriptomic plasticity of stem cell quiescence depth with age,* Joseph Palmer

P-45 Msr1 *modulates infarct volume through a collateral vessel-independent mechanism,* Leandro Prado

P-47 *The Mouse Gene Expression Database (GXD): fostering insights into the molecular mechanisms of development and disease,* Martin Ringwald

P-49 *High-throughput mouse genotyping by next generation sequencing,* Ed Ryder

P-51 *Mechanisms of bacterial tolerance in the Collaborative Cross genetic reference mouse population,* Kristin Scoggin

P-53 *The Loss-of-Function Intolerant* Actr2 *Gene is Resistant to Gene Targeting and Genome Editing,* Amy E Siebert

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Trainee Symposium Presentations

TS-1, P-11: Whole Genome Sequence Analysis Issues Revealed by Evaluation of a Linkage Peak in a Dominant ENU Suppressor Mouse Line

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We previously established a large multigenerational mouse line harboring a dominant, highly penetrant (72.4%) ENU-induced mutation that suppresses the $F5t^{m2Dgi}/F5^{tm2Dgi}$ $Tfpi^{tm1Gjb}$/+ perinatal lethal blood clotting (thrombosis) phenotype. We mapped this putative suppressor mutation to a 7.5Mb region on Chromosome 3. This region contains the essential thrombosis gene *F3*. We previously demonstrated that *F3* haploinsufficiency potently suppresses $F5t^{m2Dgi}/F5^{tm2Dgi}$ $Tfpi^{tm1Gjb}$/+ lethality, so an ENU-induced mutation affecting *F3* expression/function would be a prime thrombosuppressor candidate. However, whole exome sequencing (WES) revealed no coding mutations in *F3* or any other gene in the candidate interval. In addition, exomic mutations elsewhere in the genome did not exhibit a significant segregation with the lethal phenotype. This suggests an uncharacterized, non-exomic *F3* regulatory mutation as the thrombosuppressor. To identify candidate variants, we performed whole genome sequencing (WGS) on five $F5t^{m2Dgi}/F5^{tm2Dgi}$ $Tfpi^{tm1Gjb}$/+ mice from our line. WGS data were aligned to the C57BL/6J reference genome and variants called using an established bioinformatics pipeline. We identified 31 candidate variants within the linkage peak region: 24 SNPs and 9 small indels. None of the variants were exomic, consistent with our previous WES analysis. Of the 31 variants in the 7.5Mb region, Sanger re-sequencing analysis revealed 8 of these variants as miss-calls and the remaining 23 were unable to be re-sequenced due to the highly repetitive nature of the DNA sequences. This high number of false-positive calls possibly results from misalignment of the mixed strain background to the reference genome. The inability of our WGS analysis to identify the thrombosuppressor could potentially be due to false-negative calls resulting from misalignment, and this situation could be exacerbated by short read sequencing. We are presently exploring different WGS analysis options, including *de novo* genome assembly, to minimize misalignment issues and enable us to identify the thrombosuppressor mutant.

TS-2, P-29: Identification and Characterization of Transposable Elements in Genetic Reference Populations

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A large fraction of eukaryotic genomes, including those of mammals, consists of transposable elements (TEs). Spontaneous TE insertions also cause deleterious mutations, and drive chromosome evolution. It is difficult to identify, map, characterize, and determine the zygosity of TEs using current high-throughput short-read sequencing data. Existing approaches search for TEs by aligning billions of mostly irrelevant short reads to either a reference genome or a TE sequence library. These methods are computationally slow, have high false negative rates, and are unable to determine the TE's genomic context and/or zygosity status. Here we present a new msBWT-based TE identification and characterization pipeline that significantly outperforms previous methods in each one of these areas. We apply this method to two different laboratory mouse populations, the Collaborative Cross and a well defined set of commercially available substrains. In each population, we are able to detect fixed, shared and private TEs. We consider private TEs as those whose presence differs between individual samples with identical haplotypes, and these TEs tend to segregate in the relevant population. Thus we conclude that most private TEs represent *de novo* insertion events. We have identified hundreds of private TEs using our approach, we provide preliminary evidence that the number of private TEs depends on genetic background and that private TEs are more deleterious than either shared or fixed TEs. We will discuss the implications of these findings in classical genetic analyses and their impact on rigor and reproducibility.

TS-3, P-14: A mouse translocation dysregulating topologically – associating *Galnt17* and *Auts2* genes associated with behavioral and neuropathological phenotypes related to the human AUTS2 syndrome

Chih-Ying Chen*, P Anne Weisner, Younguk Sun, Jae Eun Yoo, Wei-Chun Kao, Huimin Zhang, Joseph M Troy, and Lisa Stubbs

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Many studies have shown human diseases can be caused by mutations in non-coding genomic regions, including promoters, intergenic enhancers, and other elements that regulate transcription of nearby genes. These elements and the genes they regulate are organized into topologically associating domains (TADs), with elements within the TADs potentially able to regulate multiple genes. Here, we describe a mouse model, 16Gso (T(5:8)16GSO), carrying a chromosomal translocation between Chromosomes 5 and 8 and exhibiting a complex suite of morphological and behavioral phenotypes. We mapped the translocation breakpoint between two genes, *Auts2* and *Galnt17*, located together within a conserved TAD, and show that the two genes expressed together in the same neuron and glial populations in developing brain. *AUTS2* region mutations have been linked to a wide variety of neurodevelopmental and neuropsychiatric diseases, and most studies have focused on *Auts2* functions. However, most mutations are genomic rearrangements, raising the potential for involvement of *Galnt17*. We found that 16Gso mice express many symptoms related to those in human AUTS2 syndrome patients including craniofacial abnormalities, developmental delay, learning deficits, repetitive behaviors, and an abnormal response to novelty; the mutants also display specific neurodevelopmental pathologies, and neurons in culture show related cellular defects. RNAseq experiments confirmed that both *Auts2* and *Galnt17* are dysregulated in 16Gso mutants, indicating that both genes may contribute to the mutant phenotypes. We are examining the role of each gene in these phenotypes using genetic complementation, and working to decipher relationships between noncoding elements and genes within the *Auts2-Galnt17* TAD. These studies will provide novel insights to the genetic and molecular basis of the wide range of phenotypes associated with *AUTS2*-region mutations, and a new model for TAD-linked multigenic disorders.

TS-4, P-64: Genomic signatures of age-dependent hybrid male sterility in the mouse

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Hybrid male sterility (HMS) is a reproductive barrier that restricts gene flow between two subspecies of mice, *Mus musculus musculus* and *M. m. domesticus*. Two major loci have been previously linked to HMS in laboratory crosses, but we observed wide variation in fertility and reproductive traits among hybrids with identical genotypes at those loci. We characterized reproductive trait variation in a panel of hybrid males bred by crossing musculus-dervied PWK/PhJ strain females to males from four inbred mouse strains of primarily *domesticus* origin. These hybrids displayed three distinct trajectories of fertility: complete sterility, complete fertility, and age-dependent fertility. Males that displayed age-dependent HMS were fertile between 15-35 weeks of age with moderate penetrance. These results point to multiple segregating HMS modifier alleles, some of which have an age-dependent mode of action. Whole-testis gene expression patterns distinguished the three fertility trajectories and implicated key regulatory pathways involved in changes to fertility with age. A subset of genes also displayed differences in allelic bias between types of hybrids. Allele-specific gene expression could inform the molecular mechanisms of HMS in mice.

TS-5, P-2: Investigating genetic diversity of centromeres: a repetitive, non-coding, unassembled region of the genome

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An enduring biological puzzle is why many genetic loci with conserved biological functions are rapidly evolving at the sequence level. We are investigating this apparent paradox using mammalian centromeres as a case-study.

Centromeres are composed of large tandem alpha satellite repeat arrays that serve as binding motifs for the centromeric histone proteins that epigenetically specify these critical chromosomal domains. Centromeres provide a platform for the assembly of the kinetochore machinery that drives chromosome segregation during cell division. Despite their well-conserved functional roles, there is remarkable centromere sequence variability between species, between individuals, and even among chromosomes in a genome. Due to the satellite repeat composition of these loci, they remain gapped on every high-quality mammalian genome assembly. The absence of centromere reference sequence hampers efforts to elucidate the full scope of diversity across these regions, infer mechanisms of their evolution, and link genetic variation across these regions to specific functional consequences.

We are cataloging diversity across centromeric sequences in genetically diverse house mice (*Mus musculus*) using a two-pronged approach. First, we are harnessing the site-specificity of CRISPR gene editing to excise single centromere containing fragments from the genome, followed by isolation for targeted long read sequencing using pulse-field gel electrophoresis. Second, we are combining publicly available mouse genome data with a k-mer based computational strategy to bioinformatically define the repeat composition of centromeres. By determining the sequence of centromeres from diverse mouse strains and subspecies, our work stands poised to shed light on the mutational mechanisms by which diversity across these regions emerges, uncover the evolutionary processes that drive their rapid evolution, reveal their roles in chromosomal rearrangements genome evolution, and facilitate functional tests of their effects on cancer, infertility and meiotic drive.

TS-6, P-13: Cellular systems genetics implicates KRAB zinc finger proteins in mediating variation in chromatin state in pluripotent stem cells

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Chromatin state modulates genome function permitting genetic information to be precisely interpreted at specific times throughout development. Determining how chromatin state is established is critical in understanding the mechanisms controlling specification and maintenance of cell identity. How genetic variation impacts chromatin regulation during development, leading to downstream changes in gene expression and ultimately phenotypic variation, is poorly understood. To address these questions we have taken a cellular systems genetics approach to early development using mouse embryonic stem cells (mESCs) as a model system. We derived a mapping panel of mESCs from 33 BXD recombinant inbred lines originating from a cross between C57BL/6J (B6) and DBA/2J (D2) mouse strains. Measuring chromatin accessibility (ca) and gene expression (e) profiles in this mapping population grown in conditions maintaining the ground state of pluripotentcy, we identified large-scale cis and trans quantitative trait loci (QTL). Many eQTL and caQTL map to six major distal loci, indicating a common regulatory system driving changes in chromatin and gene expression. Interestingly, most distal QTL correspond to locations of genes encoding KRAB zinc finger proteins (KRAB-ZFPs). We observe differential expression of transposable elements (TEs) between B6 and D2 mESCs, which are known targets of KRAB-ZFPs. Importantly, in the BXD mESCs, the genetic control of many TEs map back to the same caQTL. Current effort is focused on validating KRAB-ZFPs and associating functional elements regulated by caQTL with TE integration and nearby eQTL. Together these data support a model of evolutionary conflict between TE integration and divergence in the KRAB-ZFPs that together drive the re-wiring of gene expression networks in ESCs.

TS-7, P-34: Inter-individual variability in epigenetic and genotoxic responses to 1,3-butadiene in a population-based Collaborative Cross mouse model

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1,3-Butadiene (BD) is a known human carcinogen that is both an occupational and environmental health hazard. Although it is well-established that genotoxicity is the key mechanism of BD carcinogenesis, epigenetic events have also been observed. Previous studies in a multi-strain mouse model revealed that inter-strain (e.g. inter-individual) differences exist in both BD-induced DNA damage and epigenetic effects. These studies indicated that variation in epigenetic alterations could drive the inter-individual susceptibility to BD genotoxicity. In the present study, we investigated whether or not there is population variability in epigenetic alterations and genotoxic effects in response to BD exposure by using the Collaborative Cross (CC) mouse model. We tested thehypothesis that there are inter-individual differences in BD-induced epigenetic events and DNA damage. Male mice from 50 CC strains were exposed to 0 or 625 ppm of BD by inhalation (6 hr/day, 5 days/week) for 2 weeks. We evaluated genotoxic and epigenetic effects of BD in tissues that are a target (lung and liver) and non-target (kidney) of BD-induced carcinogenesis. Genotoxicity was assessed by measuring THB-Gua adduct levels. We observed that exposure to BD resulted in variable levels of THB-Gua adducts between strains and tissues. In order to investigate the epigenetic effects, we evaluated the levels of histones H4K20me3, H3K27me3, H3K9me3, H3K9ac, and H3K27ac in the livers. We observed variable responses in a strain-specific manner for all histone modifications as a result of BD exposure. Additionally, we analyzed the status of these histone modifications in the livers of unexposed mice and found that the strains with low levels of THB-Gua adductsafter exposure to BD were characterized by a markedly high histone H3K27ac/H327me3 ratio, a marker of transcriptionally active chromatin. In contrast, this ratio was substantially lower in strains with high levels of THB-Gua adducts.This indicates thatstrain-dependent variability of BD-induced DNA damage and potentially greater tissue susceptibility to carcinogenesis may be predetermined by the pre-exposure epigenome status of a target organ.

TS-8, P-43: Evaluating the transcriptomic plasticity of stem cell quiescence depth with age

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The long-term maintenance of somatic stem cells is in part dependent upon the cellular state of quiescence. In this study we investigate how underlying transcriptional changes in the quiescent program contribute to age-related stem cell inactivation. Taking advantage of the well-defined cyclical periods of stem cell activation and dormancy during the murine hair cycle, pure populations of melanocyte stem cells (McSCs) were isolated from birth, breeding age, and 2-year-old C57BL/6J for whole genome gene expression analysis. The results show global transcriptional reprogramming, as actively dividing stem cell precursors colonize the hair follicle niche and transition into the quiescent state. We first define a core *in vivo* gene expression network of quiescent McSCs, and then evaluate changes in the regulatory mechanisms resulting from the prolonged quiescence experienced by these cells with age. Interestingly aged McSCs predominantly express a reinforced quiescent signature, with an overall reduction in translation and increased expression of genes associated with regulation of homeostasis and adhesion. This suggests that McSC quiescence is not a singular state and, with age, at least a portion of McSCs exist in a deeper level of quiescence. Using *in vitro* quiescent culture conditions, active-, 2- and 4-day quiescent cells were analyzed to confirm how increasing lengths of quiescence in cells of the melanocyte lineage affects their ability to reactivate. We propose, that similarly to what we observe *in vitro,* McSCs become refractory to activation signaling due to the increased depth of quiescence resulting from extended quiescence with age. Informed by our previous transcriptomic analysis we have begun identifying biomarkers associated with changes in depth of quiescence *in vitro* to confirm this process *in vivo*. Our future direction is to further investigate the mechanisms governing deep quiescent stem cell populations and how they can be targeted for tissue regeneration interventions in aged organisms

TS-9, P-48: Discovering Genetic Modifiers in a new Mouse Model of Niemann-Pick Disease, Type C

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Niemann-Pick Disease, Type C (NPC) is a fatal neurodegenerative disorder that exhibits intracellular accumulation of unesterified cholesterol in late endosomes/lysosomes and marked accumulation of glycosphingolipids in neuronal tissue. These subcellular pathologies eventually lead to phenotypes of hepatosplenomegaly and neurological degeneration leading to premature death. NPC disease is extremely heterogeneous in the timing of clinical presentation (prenatal to adulthood), is associated with a wide spectrum of causative *NPC1* mutations, and the time of onset or severity of the disease shows little concordance with the predicted consequences of *NPC1* gene mutation on protein function. Currently there are no FDA-approved therapies that effectively increase lifespan or slow disease progression.

To further explore the influence of genetic background in NPC1 severity and mapping potential genetic modifier(s), we generated a mouse model for NPC harboring a novel allele ($Npc1^{em1Pav}$ aka $Npc1^{S1062_I1064del}$) using CRISPR/Cas9 on a C57BL/6J background. By using speed congenics we established N4 intercross mutants with BALB/cJ with a significant ($p<0.0001$) increased lifespan (84.3 + 5.25 days) compared with the original C57BL/6J colony (69.7 + 4.36). Backcross N2 $Npc1^{S1062_I1064del}$ homozygous mutants also showed a significant increase in lifespan (78.36 + 6.90) with greater variance suggesting strain-specific modifiers influencing NPC1 disease severity.

Using the GigaMUGA genotyping array we analyzed N2 mutants (N=202) and detected significant linkage to markers on Chromosome 1 (LOD=5.57) and Chromosome 7 (LOD=8.91). To follow up on these results we are currently integrating linkage data, RNA seq and WES data from an NPC1 patient cohort with the hopes of prioritizing candidate genes for *in vitro* and *in vivo* validation. Identification of modifiers will contribute to our understanding of the highly variable phenotype observed in NPC patients and advance our efforts to improve patient diagnosis and therapy.

TS-10, P-53: The Loss-of-Function Intolerant *Actr2* Gene is Resistant to Gene Targeting and Genome Editing

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Susceptibility to venous thromboembolism (VTE) is increased by inheritance of Factor V Leiden ($F5^{L}$), however, only ~10% of people inheriting $F5^{L}$ develop VTE, illuminating the need to identify genes that modify VTE risk. We recently reported a dominant, p.R258G missense mutation in the *Actr2* gene ($Actr2^{MF5L12}$) as a major thrombosuppressor of perinatal lethal thrombosis in mice homozygous for $F5^{L}$ ($F5^{tm2Dgi}$) and heterozygous for tissue factor pathway inhibitor ($Tfpi^{tm1Gjb}$). The *Actr2* gene encodes ARP2 actin-related protein 2, which is an essential member of the Arp2/3 complex. Arp2/3 is ubiquitously expressed and is necessary for actin cytoskeletal regulation in all eukaryotic cells, including platelets. We recently attempted to generate independent R258G missense and knockout alleles in mice using CRISPR/Cas9 genome editing. The failure to produce any mice carrying the *Actr2-* allele suggested that even heterozygosity is incompatible with embryonic survival. To further investigate this unexpected finding, ES cells (Clone:EPD0727_2_H12) containing the targeted $Actr2^{tm1a(KOMP)Wtsi}$ “Knockout First” allele were obtained. Transfer of 79 injected blastocysts into foster mothers yielded five males with 20-90% chimerism. Successful germline transmission occurred from only the 20% chimera. *Actr2* locus targeting was confirmed by 5’ and 3’ long range PCR from tail DNA in the four non-transmitting chimeras. However, long range PCR analysis of the 20% chimera and its progeny revealed only mistargeting outside of the *Actr2* locus. This demonstrates that the KOMP ES cells were heterogeneous for correct/incorrect *Actr2* targeting and confirms that a 50% reduction of *Actr2* function is incompatible with survival. In summary, our attempts to generate *Actr2-* mice illustrate the importance of meticulously screening for correct/incorrect vector incorporation in gene targeting experiments. This is especially relevant for loss-of-function intolerant genes such as *Actr2*, because targeting this subset of genes results in negative selection.

TS-11, P-15: Alterations in metabolism and circadian rhythm in a *Pax6* mutant mouse line

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The transcription factor paired box protein 6 (PAX6) is a pleiotropic factor that is involved in the development of brain, central nervous system, eye and the pancreas. Its role in maintenance of pancreatic islet homeostasis and metabolism has been a subject of a few recent studies. Interestingly, PAX6 is not only involved in the development of the pineal gland, the main regulator of diurnal rhythm, but several patients carrying *PAX6* mutations show hypoplasia or absence of the pineal gland. Therefore, such patients may present several phenotypes including pineal gland agenesis. However, much less is studied in this regard, possibly due to such features being masked by more conspicuous diseases such as overt diabetes and eye defects.

Here, we investigated an ENU-generated *Pax6* mutant mouse line, which displays eye defects and metabolic alterations. Most strikingly, homozygous mutant mice failed to display metabolic flexibility and showed a lack of typical diurnal changes in metabolic rate, strongly indicating altered circadian rhythm. This was further accompanied by changes in feeding behavior, where mutant mice consume more food during the inactive phase as compared to their wild type littermates. Moreover, we found changes in the expression of circadian genes such as *Clock*, *Nr1d1* and *Per1* in the liver, suggesting altered physiology may arise from disturbances in activation and repression cycles. Furthermore, presence of the pineal gland was observed in homozygous mutant mice however, defects in the eye suggest a compromised visual pathway that may also affect non-visual responses such as sleep regulation and entrainment of circadian rhythm. Hence, this study aims to illuminate an interaction between circadian rhythm and metabolism with regards to the function of PAX6.

TS-12, P-18: Pairing systems genetics and longitudinal analyses to identify regulators of complex lung disease phenotypes

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Mouse models of lung disease have been instrumental to our understanding of pathogenesis and have important roles in addressing existing unmet therapeutic needs. Given that many lung diseases are heterogeneous and driven by numerous genetic and environmental factors, the use of multi-parental mouse populations to facilitate robust identification of mechanisms regulating such complexity is warranted. We employed a systems genetics approach with the Collaborative Cross mouse population to overcome existing challenges in elucidating the molecular mechanisms driving airway remodeling in the context of asthma. Airway remodeling refers to alterations to the cellular and tissue composition of the airways that often cannot be fully resolved and may contribute to asthma attack fatality. We chronically exposed 31 Collaborative Cross (CC) strains and BALB/cJ mice to house dust mite allergen to induce features of airway remodeling that mirror human disease. CC strains exhibited a range of responses to chronic allergen exposure as determined by airway inflammatory profiles, airway mucus content, and quantitative and semi-quantitative morphological analysis of airway tissue for goblet cell metaplasia, subepithelial fibrosis, and smooth muscle hyperplasia. Of note, the correlations among these phenotypes indicated both distinct and shared genetic regulation of disease traits. RNAseq of isolated airway tissue will provide insight on the transcriptional patterns underlying disease severity, aid in prioritizing candidate phenotypic regulators, and be used to determine how mouse model transcriptional profiles align with existing human disease samples. A subset of CC strains will be selected for a time course of allergen exposure to investigate the signaling events controlling remodeling initiation and progression. This dataset will be the foundation for robust airway remodeling analyses that are currently intractable in the human population and are critical steps in identifying potential therapeutic targets.

TS-13, P-54: Variable effects of steroid treatment on patient derived keloid fibroblasts – is underlying genetic/epigenetic heterogeneity the cause?

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Keloid lesions are fibrotic scars on the skin that occur in susceptible individuals due to abnormal wound healing and are characterized by excessive collagen production. Certain populations, such as African Americans and Asians have a much higher incidence of keloids, suggesting a strong genetic or epigenetic component that contributes to this disease. Due to their high recurrence rates following surgical excision, keloids are one of the most challenging dermatological conditions to effectively treat. Although there is no universally accepted treatment for keloids, intralesional corticosteroids are commonly used as first-line therapy to aid in softening and flattening keloids by diminishing collagen synthesis and inhibiting keloid fibroblast proliferation. Corticosteroids are also used as adjunctive therapy to surgical excision and have demonstrated a reduction in recurrence rates. However, some reports in the literature suggest that a significant number of keloid patients may be refractory to steroid therapy, with some patients exhibiting a worsening of their keloids following steroid therapy. Remarkably, these findings have been replicated *in vitro* in our laboratory using patient derived keloid fibroblasts that exhibit highly variable cell proliferation rates upon steroid treatment. Some keloid fibroblasts are sensitive to steroid treatment and exhibit attenuated proliferation, while others are either refractory or show hyperproliferation upon steroid treatment. We are now interested in uncovering the underlying molecular mechanisms that may mediate the variable effects of steroid treatment on keloids from different patients. Based on our data so far, we hypothesize that genetic and/or epigenetic heterogeneity may lead to the variable responses observed upon keloid therapy using steroids. We are currently testing our hypothesis by systematically evaluating DNA methylation and post-translational histone modifications in keloid derived fibroblasts as well as employing transcriptomic and whole genome sequencing approaches to study the underlying differences between keloid fibroblasts that are either sensitive or resistant to steroids.

TS-14, P-45: *Msr1* modulates infarct volume through a collateral vessel-independent mechanism

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Infarct volume in experimental stroke models varies across inbred mouse strains. Across most strains, infarct volume is inversely correlated with the extent of collateral vessels, which enable reperfusion of the ischemic territory. However, certain mouse strains share similar collateral vessel anatomy but exhibit significantly different cerebral infarct volumes. In a surgically induced mouse model of ischemic stroke, we previously identified a quantitative trait locus on Chromosome 8 (*Civq4*, LOD = 9.8) that contributes 21% of the phenotypic variance of infarct volume in a cross between C3H/HeJ and C57BL6/J. In this study, we created recombinant congenic mouse lines carrying different segments of the *Civq4* region from C57BL6 introgressed into the C3H background (Line C) and from C3H introgressed into the C57BL6 background (Line B). We examined infarct size pial collateral vessel anatomy in these animals and our results showed that the Line B has a similar number of collateral vessels connections and infarct volume when compared to C57BL6 animals. However, in the group Line C we observed similar number of collateral vessels connections but a larger infarct volume when compared to C3H animals. In addition, a strain-specific nonsynonymous coding variant for *Msr1*, predicted to be damaging, elevated these as candidate genes for *Civq4*. To investigate *Msr1* as candidate gene, we evaluated infarct volume pial collateral vessel anatomy in mouse knockouts for the *Msr1* gene. We observed in *Msr1*-KO animals (C57BL6-*Msr1*^{tm1Csk}) similar number of collateral vessels connections and infarct volume when compared to wild-type C57BL6 animals. However, in *Msr1*-KO animals (C3H background) we observed similar number of collateral vessels connections but a larger infarct volume when compared to wild-type C3H animals. Taken together, our data suggest that the *Msr1* gene might play a role in the neuropathology of cerebral ischemia modulating infarct volume through a collateral vessel-independent (neuroprotective) mechanism.

TS-15,P-33: Dissecting the role of TLR4 signaling during SARS- and SARS-like CoV infection in mice using the Collaborative Cross reference population and multiple mapping approaches

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Newly emerging pathogens like influenza viruses (H1N1, H5N1, H7N9) and the group 2B coronaviruses (Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), SARS-like CoV HKU3) pose a constant threat to human and animal health leading to significant morbidity and mortality. While it has been shown that the host genetic background plays an important role for the outcome after infection many critical determinants of susceptibility remain to be identified.

The established Collaborative Cross (CC) population of genetically diverse mice and the SARS-CoV as well as HKU3-CoV *in vivo* infection models have been utilized to probe host immunity in the lung. After intra-nasal infection, weight loss, viral titers, as well as lung pathology were evaluated through day four and used to perform quantitative trait locus (QTL) mapping studies.

Among others, multiple candidate genes within the TLR4 signaling pathway were identified: *Anpep* (day four SARS-CoV viral titer QTL – Chr 7); *Git2* (HKU3-CoV mortality QTL – Chr 5); *Wisp1* (day four HKU3-CoV weight loss QTL – Chr 15) and others.

It has been shown that disruption of the TLR3/4 pathway and the TLR-supported balance of the innate immune response renders mice more susceptible to SARS-CoV infection. We were able to show a more resistant phenotype for *Anpep* knock-out (KO) mice as well as a more susceptible phenotype for *Git2* KO mice after SARS-CoV infection compared to wild-type controls. *Wisp1* interacts with a plethora of steps within the TLR4 signaling pathway and the phenotype of *Wisp1* KO mice after SARS-CoV infection remains to be determined.

Using natural allelic variation in mouse models of outbred populations, our data supports the hypothesis that the TLR3/4 signaling pathway plays a pivotal role in regulating the host immune response to multiple group 2B SARS-like CoVs after infection.

TS-16, P-27: Progression of colorectal cancer through epidermal growth factor receptor (EGFR)-independent mechanisms

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Epidermal growth factor receptor (EGFR)-targeted therapies have been approved for colorectal cancer (CRC) treatment. However, previous studies have observed that efficacy of anti-EGFR therapy in humans is influenced by the genetic environment on which colonic tumors arise. Mutations in *KRAS* explain some non-responding CRCs, but even in cancers lacking *KRAS* mutations, little is known about which cancers are likely to respond to EGFR targeted treatment. In this study, we used a mouse model with a conditional *Egfr* allele, (*Egfr*^{tm1dwt} also called *Egfr*^{f}) to demonstrate the existence of EGFR-independent CRCs. We also used a unique mouse model that contains conditionally inactivated *Apc* alleles (*Apc*^{tm1Tno} or *Apc*$^{f/f}$) in combination with a conditionally activatable allele of oncogenic *Kras* (*Kras*^{tm4Tyj} *or Kras*$^{LSL\text{-}G12D}$) to assess aggressiveness of EGFR-dependent (*Apc*$^{f/f}$*, Kras* $^{LSL\text{-}G12D/+}$) versus EGFR-independent (*Egfr*$^{f/f}$*, Apc*$^{f/f}$*, Kras* $^{LSL\text{-}G12D/+}$) tumors. Following delivery of Cre recombinase-expressing adenovirus (AdCre) to the distal colon, 93% of the *Egfr*$^{f/f}$*, Apc*$^{f/f}$*, Kras* $^{LSL\text{-}G12D/+}$ mice develop adenomas. In addition, biweekly colonoscopies confirmed that colonic tumors grow faster in the absence of EGFR. Moreover, RNAseq analysis revealed a group of 32 genes that are differentially expressed in colonic tumors that grow independent of EGFR. Quantitative PCR validated the differential expression levels of several genes involved in IL10RA pathway, including *Sult1a1*, *Il10, Il10ra, Maob, Aadac,* and *Tnf*, in colon tumors without EGFR providing more evidence that EGFR-independent tumors are modulated by the effect of cytokines. These findings demonstrate the existence of an EGFR-independent mechanism by which CRC can progress. This study will advance our understanding of anti-EGFR resistance in CRC treatment, ultimately contributing to more effective therapies.

Verne Chapman Memorial Lecture

O-1: Genome-Wide Dynamics of Chromatin Modifiers

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The eukaryotic genome is stored in the nucleus as chromatin, a dynamic structure of DNA and histone proteins. Chromatin contains a vast array of features that directs how, when and where genomic DNA is made accessible by the regulatory machinery. The nucleosome is the core unit of chromatin and they are highly dynamic structures. Nucleosomes can be variable in histone protein composition, undergo a wide array of post-translational modifications, and are subject to changes in their localization at genomic sites. These variations in nucleosome biology instruct cells how to utilize epigenomic loci and the underlying DNA sequences. As a result, regulation of chromatin delivers precise instructions to cells leading to secondary processes that are physiologically critical. Thus, beyond serving as a DNA storage molecule, chromatin serves as a template that enables cells to leverage multivalent signals surrounding genomic sites as instructions to cellular machinery for biological programs. There are three categories of proteins and enzymes that modify chromatin by depositing, removing or recognizing post-translational modifications (PTMs) of the histone, categorized as writers, erasers, and readers, respectively. A fourth class of chromatin modifiers consists of ATP-dependent chromatin remodelers that regulate nucleosome units. These complexes are multi-subunit molecular machines that mobilize nucleosomes by breaking histone-DNA contacts using ATP hydrolysis. The remodelers are the subject of this presentation. Our work analyzes the regulation of assembly and genomic targeting of biochemically distinct forms of chromatin remodeling complexes and how this impacts functional and phenotypic diversity of remodelers. Specifically, the presentation will focus on changes to composition and assembly of remodeling complexes during development and disease states, and a mechanistic characterization of canonical and non-canonical remodeling complexes.

Oral Presentations

O-2: NAD Deficiency, Congenital Malformations and Vitamin B3 Supplementation

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Congenital malformations arise due to genetic and environmental factors and understanding the interplay between these in causing malformation might lead to preventative opportunities, in some cases. We are identifying genetic and environmental factor that disrupt embryogenesis in humans and mice.

In studying families with vertebral and cardiac defects we identified mutations in four cases in either of two genes: 3-hydroxyanthranilic acid 3,4-dioxygenase *(HAAO)* and kynureninase *(KYNU),* encoding kynurenine pathway enzymes. Three patients carried homozygous loss-of-function mutations in *HAAO* or *KYNU* and one patient carried compound heterozygous mutations in *KYNU*. All four patients had vertebral, cardiac and renal defects, amongst others and recurrent miscarriage was a feature in two families. Nicotinamide adenine dinucleotide (NAD) is synthesized *de novo* from tryptophan via the kynurenine pathway. NAD is also synthesized more directly from vitamin B3. The patients had reduced circulating NAD levels. *Haao* or *Kynu* null mouse embryos developed similar defects to the patients, due to NAD deficiency. In null mice NAD deficiency, malformations and miscarriage were prevented by niacin (vitamin B3) supplementation during gestation (Shi et al NEJM 2017).

O-3: Comparative genetics and genomics of mouse strains and species at Mouse Genome Informatics (MGI)

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The availability of hundreds of genetically distinct and phenotypically characterized mouse strains is at the core of the power of mouse models to detect genotype-to-phenotype and disease relationships. The recent availability of annotated genome assemblies for nineteen mouse strains and species generated by the Mouse Genomes Project further enhances the power of the mouse as a model system for investigating the genetic basis of human health and disease. The Mouse Genome Informatics database (MGI, www.informatics.jax.org) has implemented new functionality to take advantage of the availability of strain genomes including, the development of an interactive Multi Genome Viewer (MGV) and summarized mouse strain-specific data web pages. MGV allows users to display and interact with the annotated genomes all available mouse strains and species in a compact, comparative graphical interface. The new strain summary pages feature official strain names, synonyms, SNP data, known phenotypic and sequence variants and disease models, links to vendors and references. Links to external resources such as the Mouse Phenome Database for baseline strain characteristics are also provided when relevant. The new strain-centric interfaces available from MGI will allow researchers to leverage emerging large-scale phenotype and genome data sets to identify the precise genomic differences among mouse strains. MGI is funded, in part, by HG000300.

O-4: Accounting for haplotype uncertainty in QTL mapping of multiparental populations using multiple imputation

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Multiparental populations (MPP) are powerful genetic population resources descended from multiple inbred founder strains. In mice, two examples are the inbred Collaborative Cross (CC) and their outbred sister population, the Diversity Outbred (DO) stock. Descent from a larger set of founder genomes results in these populations possessing more genetic and phenotypic diversity than traditional bi-parental experimental populations, and has allowed them to be extensively used to map quantitative trait loci (QTL) for complex traits.

Rather than mapping based on observed marker genotypes, as is done in SNP association, QTL mapping in MPP is often performed through haplotype-based association, which can implicitly model unobserved variants and local epistatic interactions. The haplotype identity at a locus, in terms of descent from the founder genomes, is not directly observed and must rather be probabilistically inferred. In practice, an approximate QTL association approach is used that directly regresses the phenotype on the haplotype probabilities, whereas more rigorous statistical procedures that model or formally acknowledge the haplotype uncertainty are computationally slower. However, problematic patterns of haplotype uncertainty, in particular rare or unlikely haplotypes at a locus, can produce spurious QTL signals with the approximate procedure.

We show clear examples of this problem in samples from the CC and Heterogeneous Stock (HS) rats, and propose several statistical remedies, including a conservative multiple imputation approach, which both characterizes the uncertainty around the statistical score of QTL association, as well as stabilizes it. We further demonstrate how to evaluate and detect signs that haplotype uncertainty is potentially inflating the association at a potential QTL. These approaches should help researchers better focus and prioritize their valuable resources for following up QTL.

O-5: The dynamic transcriptional landscape of mammalian organogenesis at single cell resolution

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During mammalian organogenesis, the cells of the three germ layers transform into an embryo that includes most major internal and external organs. The key regulators of developmental defects can be studied during this crucial period, but conventional approaches lack the throughput and resolution to obtain a global view of the molecular states and trajectories of a rapidly diversifying and expanding number of cell types. Here we set out to investigate the transcriptional dynamics of mouse development during organogenesis at single cell resolution. With an improved single cell combinatorial indexing-based protocol ('sci-RNA-seq3'), we profiled over 2 million cells derived from 61 mouse embryos staged between 9.5 and 13.5 days of gestation (E9.5 to E13.5; 10 to 15 replicates per timepoint). We identify hundreds of expanding, contracting and transient cell types, many of which are only detected because of the depth of cellular coverage obtained here, and define the corresponding sets of cell type-specific marker genes, several of which we validate by whole mount *in situ* hybridization. We explore the dynamics of proliferation and gene expression within cell types over time, including focused analyses of the apical ectodermal ridge, limb mesenchyme and skeletal muscle. With a new algorithm (Monocle 3), we identify the major single cell developmental trajectories of mouse organogenesis, and within these discover examples of distinct paths to the same endpoint, *i.e.* branching and convergence. These data comprise a foundational resource for mammalian developmental biology, and are made available in a way that will facilitate their ongoing annotation by the research community.

O-6: Exploring rodent genetic and phenotypic diversity for biomedical and environmental applications

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The order Rodentia underwent an extraordinary adaptive radiation during the Cenozoic and accounts for nearly half of all known mammalian diversity containing over 2,000 species. Rodents spread over almost every landmass on earth and occupy almost all terrestrial ecosystems from rainforests, deserts, and arctic tundra. Murid rodents have a rate of chromosomal rearrangement that has been estimated to be between three times and hundreds of times faster than in primates. The inbred laboratory mouse, C57BL/6J, was the second mammalian genome after human to be fully sequenced, underscoring the prominence of the mouse as a model for mammalian biology. However, genome sequences are only available for less than 60 rodent species, many of which are scaffold level draft assemblies.

Advances in second and third generation sequencing have made it possible to generate chromosome scale genome sequences. As part of the Vertebrates Genome Project, we are sequencing genomes of several distinct rodent species groups with potential biomedical and climate change applications. For example, the Algerian mouse is nocturnal, and can drink two-thirds less water and can stand much higher temperature than the domestic mouse. *Acomys* (African spiny mice) can shed their dorsal skin as a deterrent to avoid predators and fully regenerate the lost tissue without fibrosis or tissue overgrowth. *A. russatus* is of particular interest for adaptive studies as it thrives in the desert and can ingest seawater while still maintaining kidney function. Within the *Onychomys*, the Southern Grasshopper Mouse (*O. Torridus*) is insensitive to one of the most painful stings in the animal kingdom, the bark scorpion, having evolved the ability to block voltage-gated pain transmission. We will present our initial genome sequences, and show examples of novel genetic adaptations in genes associated with pain, regeneration, and aridity.

O-7: Targeted investigation of PhyloCSF regions identifies more than 100 novel protein-coding genes on the mouse reference genome

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We produce the Ensembl/GENCODE reference genesets for human and mouse as part of the Ensembl project, and a key focus of our current efforts is to provide more accurate annotation of coding sequences (CDS). Here, we will discuss the creation of a workflow that allows us to identify prospective novel CDS in mouse based on evolutionary analyses using PhyloCSF (Phylogenetic Codon Substitution Frequencies) and comparative annotation of loci in human and mouse. PhyloCSF supports CDS annotation based on the alignment of multiple genome sequences and determines whether a given alignment is likely to represent a functional, conserved protein-coding sequence by determining its likelihood ratio under coding and non-coding models of evolution. We generated PhyloCSF datasets across 6-frame theoretical translations of the human and mouse reference genomes and used machine learning algorithms to identify and prioritize candidate novel coding regions. We manually investigated more than 1000 of the top scoring PhyloCSF regions in mouse and human and added ~130 protein-coding genes to the mouse GENCODE geneset, the majority of which are not represented in any way in other reference databases such as RefSeq and Uniprot. The PhyloCSF regions generated by the project are freely available as a trackhub in the Ensembl and UCSC genome browsers and all Ensembl/GENCODE gene annotation is also accessible via the Ensembl and UCSC browsers and Ensembl FTP site and Perl and REST APIs.

O-8: Background-dependent mutation rate and strong deleterious effects of *de novo* transposition of endogenous retrovirus in mouse inbred strains

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The explosive growth in the number of individuals with available whole genome sequence (WGS) has reinvigorated the field of transposable element (TE) biology. TEs represent a large fraction of the murine genome. Some TE insertions, such as the one at the *agouti* locus, have well known and dramatic phenotypic effects and recent studies demonstrate that the complement of TEs is variable among laboratory strains. However, many fundamental questions remain unanswered, including the mutation rate, the spectrum of functional effects of *de novo* TEs and their impact on rigor and reproducibility of mouse studies. We have taken advantage of our unique resources/tools to address these important questions. These resources/tools include 1) a newly developed computational pipeline to identify, map and characterize TEs, 2) WGS of many related individuals within well-defined pedigrees and 3) the ability to test the effect of *de novo* TE insertions in both gene expression, and ultimately on fitness. We conclude that the rate of mutation for a given TE family varies dramatically among inbred strains. Mutation rates are highly consistent among substrains of a given strain-group, such as BALB/c and C57BL/6, independently of their commercial and historical origin. This observation, coupled with the substantial variation in the number of *de novo* TEs observed among CC strains, strongly supports the conclusion that genetic background represents a key factor in mutation rate. In addition, almost half of *de novo* TEs are deleterious as demonstrated by the statistically significant differences in the pattern of insertions (exon, intron and intergenic) relative to ancestral neutral TEs, their rapid purge from populations, the departure from HW equilibrium, the causal effect on expression of nearby genes and the detection of TE/gene chimeric transcripts. Our results indicate that in some inbred strains *de novo* TEs can be a substantial source of phenotypic variation and may impact reproducibility of results.

O-9: Genetic variation influences ground state pluripotency in mouse embryonic stem cells through a hierarchy of molecular phenotypes

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The influence of genetic variation on differentiation propensity of human pluripotent stem cell lines has important consequences for regenerative medicine and the study of early development. Mouse embryonic stem cells (mESCs) grown under controlled conditions occupy a ground state where pluripotency-associated transcriptional and epigenetic circuitry are highly active. We observed variability in expression of core pluripotency genes in genetically diverse mESCs grown in media lacking inhibitors that reinforce the ground state. To dissect the genetic basis of this variation in stability of ground state pluripotency, we conducted genetic analysis of gene expression and chromatin accessibility in mESCs grown under sensitized conditions (LIF + GSK3 inhibitor). We profiled gene expression and chromatin accessibility in 185 lines derived from genetically heterogeneous Diversity Outbred mice. We mapped thousands of loci that affect chromatin accessibility (caQTL) and/or transcript abundance (eQTL). We found eleven instances where distant QTL co-localized in clusters, suggesting a common regulator. One locus on Chromosome 15 controlled the expression of 208 genes including many with known roles in maintenance of pluripotency. We applied causal mediation analysis and identified *Lifr* (leukemia inhibitory factor receptor) transcript abundance as the causal intermediate for these eQTL. Moreover, a joint mediation analysis of gene expression with chromatin accessibility yielded a single peak upstream of *Lifr* containing one SNP with a strain distribution pattern matching founder haplotype effects on *Lifr* downstream targets. Functional assays confirmed that *Lifr* genotype influences self-renewal capacity in an orthogonal set of mESCs derived from recombinant inbred lines. Furthermore, allele swap experiments in mESC lines with opposing alleles upstream of *Lifr* verified that the SNP we identified strongly influences mESC pluripotency. Thus, we detected a causal chain of molecular events: a single SNP modulates regulatory element accessibility, which affects *Lifr* expression, leading to large-scale transcriptional shifts including known and novel pluripotency-associated genes. These results reveal mechanistic details underpinning variability in stability of ground state pluripotency in mESCs, highlight the power of diverse mouse populations for dissecting molecular regulatory networks, and represent a resource for the study of molecular events driving early development.

O-10: Updating The Mouse Genome: The Impact of Mutation and Drift on C57BL/6J

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The inbred mouse strain C57BL/6J is arguably the most widely used animal model in biomedical research and is often referred to simply as "the mouse". The first high quality draft of the mouse genome sequence, reported in 2002, was based on the assembly of sequenced BAC clones from several female C57BL/6J mice whose provenance is poorly understood. The mouse reference genome has been actively curated and revised with major updates and patches culminating in the current release version GRCm38.p6. In parallel, the C57BL/6J strain has been maintained by brother-sister mating at the Jackson Laboratory (JAX) since at least 1948. However, it was not until 2003 that JAX established its genetic stability program to effectively reduce the impact of genetic drift. At that time, the C57BL/6J strain was re-established from a single breeding pair, "Eve" and "Adam". Efforts to obtain the whole genome sequence of Eve are reported elsewhere. Here we describe whole-genome, short-read sequencing of 24 pedigreed descendants of the Eve and Adam spanning generations G4 through G11. Analysis of these data revealed tens of thousands of fixed differences between the current reference genome and actual C57BL/6J mice. These include SNVs, small indels, and short tandem repeat-length variants, some of which are predicted to have potential functional consequences. However, we found no evidence of active transposable elements. We developed a statistical model to estimate the mutation rate in a setting where actual mutations are needles in a haystack of sequencing errors. We find that the C57BL/6J genome is remarkably stable with unusually low mutation rates. Nonetheless, change happens. Discrepancies between the reference genome and the genetic makeup of the mice it purports to represent can result in errors of interpretation and wasted effort for scientists working with C57BL/6J mice. Therefore we have proposed numerous updates for the upcoming release of the mouse reference genome to bring it back in line with the actual genome of "the mouse".

O-11: Animal Modeling in the Era of Precision Medicine

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Comparative medicine and animal modeling allow us to understand genetics, disease, and basic biology across species. Because genome editing can be performed in a quick and reliable manner in a variety of species using CRISPR/Cas9, this technology is transforming the ability to generate appropriate animal models to study human disorders. Genetically engineered animals can be used effectively to dissect out various aspects of human disease, including identification of putative disease genes, re-creation and validation of potential disease-causing alleles observed in patients, and testing of targeted therapeutics. Our recent studies to determine the genetic basis of cystic kidney disease in human patients illustrate the power of using CRISPR/Cas9 to generate informative animal models to validate the disease-causing nature of rare human variants. Importantly, the ability to use CRISPR/Cas9 technology effectively in a wide variety of species expands the choice of model organism such that the most appropriate animal species can be used to address the specific scientific question being asked.

O-12, P-22: Identification of meiotic non-crossovers in mice

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Programmed DSBs induced by SPO11 are required for successful pairing of homologous chromosomes in meiotic prophase I. Their position is mostly determined by the binding affinity of the zinc-finger methyl-transferase protein PRDM9, trimethylating H3K4 and H3K36. All DSBs need to be repaired, either by crossover (CO, with an exchange of chromatid arms), or by non-crossover (NCO, with no exchange), both being accompanied by gene conversions. Even though NCOs are more frequent (90%) than COs (10%), their genome-wide/chromosome-wide detection has not been shown yet in mice.

We detected ~100 of NCO gene conversions on ten mouse autosomes using WGS of corresponding C57BL/6J-Chr #PWD chromosomal substitution strains and their progenitors C57BL/6J (B6) and PWD/PhJ (PWD). This model combines the benefits of high diversity of the *Mus musculus domesticus* and *Mus musculus musculus* genomes and the 10-fold accumulation of gene conversion events during construction of a chromosome substitution strain. Based on the converted and surrounding non-converted SNPs we were able to identify gene conversions shorter than 100 bp as well longer than 200 bp.

We found PRDM9^{B6} and PRDM9^{PWD} binding motifs within 250 bp from the NCO gene conversions. Additionally, NCOs overlapped the known H3K4me3 modifications and DMC1 protein peaks from the ChIP-seq data of B6, PWD, (B6xPWD)F1, (PWDxB6)F1 genotypes, proving the meiotic origin of the observed gene conversions. A comparison of the origin of NCOs supported the idea of the asymmetry of PRDM9 binding. The gene conversions led to erosion of the PRDM9 binding sites and to a GC bias ($P < 0.001$). Interestingly, the GC bias was also present in the surrounding 800 bp, suggesting the presence of historical recombination.

O-13: The biological importance of the unique DNA binding properties of MECP2

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DNA methylation of cytosine bases (mC) is an epigenetic mark critical for regulating gene expression, imprinting and X chromosome inactivation in mammals. It most frequently occurs in the symmetrical CG context. This mark is read by four proteins with shared evolutionary origin (MECP2 and MBD1/2/4) via their methyl-CpG binding domains (MBDs). The amino acid sequence of MBDs is similar though not identical between family members, but regions outside the DNA binding domains are unrelated and interact with diverse protein partners. We focus on MECP2, which primarily functions to repress transcription via recruitment of the NCoR/SMRT co-repressor complex. It is essential for proper neuronal function, as loss-of-function mutations result in the profound neurological disorder, Rett syndrome. Recent studies have shown that the MECP2 MBD is uniquely able to bind to mCAC, as well as to canonical mCG sites. To test the hypothesis that this is of functional importance given the high levels of mCAC in the brain, we replaced the MBD of MECP2 with that of MBD2 in knock-in mice. These mice display adverse neurological phenotypes from weaning, resulting in premature death. We conclude that the unique DNA binding properties of MECP2 are essential for its function.

O-14: Intestinal Tumors in Hybrids from Crosses of Collaborative Cross Mice with a Sensitized Strain Reveal an Array of Phenotypes

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Stomach, small intestine, colon, and rectal cancers affect hundreds of thousands of people worldwide each year. Gastrointestinal (GI) cancer initiation, growth, and progression depends on both environmental and genetic factors. The Adenomatous Polyposis Coli *(APC)* gene is one of the top 5 genes somatically-mutated in GI cancers. Germline mutations in the *APC* gene cause an autosomal dominant disorder, Familial Adenomatous Polyposis (FAP). FAP patients develop hundreds to thousands of adenomas, mostly in the colon, which if left untreated progress to cancer. Mouse models serve as useful tools to study cancer. The Collaborative Cross (CC) strains were developed as a powerful resource to dissect genetic factors affecting complex disorders. We designed a screen to determine whether the genomic diversity in selected CC strains could result in novel GI tumor phenotypes. Females from different CC strains were mated with males from the sensitized, congenic 129.B6 Apc^{Min} $Atp5a1^{Mom2R}$ /+ + line. This line was chosen because it develops few adenomas and has a long life span due to modifier loci suppressing tumorigenesis in the 129 background. We also anticipated that adenomas would have a higher probability of progressing to adenocarcinomas in long-lived mice. Hybrid F1 offspring were aged (250+ days of age) and the entire GI tract (from stomach to rectum) was evaluated for tumor phenotypes, including location, number, shape and size. The results reveal a diversity in GI tumor phenotypes, which develop due to the unique combinations of alleles in the CC strains and the congenic line. This exploratory work builds a foundation for future studies to identify new modifier genes that can effectively suppress polyposis, even in the presence of a dominant mutation in a tumor suppressor gene. Research supported by NCI R21 CA202496 to LDS.

O-15: PRDM9 functions as a specialized meiotic pioneer factor that activates recombination hotspots by recruiting a nucleosome remodeling factor and opening chromatin

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In eukaryotes, DNA is packaged with histones to form nucleosomes, the basic unit of chromatin. Because genetic information is occluded by chromatin, regulation of DNA accessibility is essential for normal cellular development, identity, and function. Pioneer factors represent a special class of DNA binding proteins that overcome chromatin barriers. To date, the study of pioneer factors has focused on lineage-specific gene regulation; however, the role of pioneer factors in other aspects of chromatin biology has not been explored. Here we identify PRDM9 as a specialized pioneer factor in regulating chromatin remodeling at meiotic recombination hotspots. We find that recombination hotspots are newly-accessible sites of open chromatin in early meiotic prophase I. DNA accessibility at hotspots is PRDM9-dependent and occurs in the absence of meiotic DNA double strand breaks (DSBs), showing that chromatin accessibility occurs prior to and independent from DSBs, unlike somatic cells. Additionally, we find that recombination hotspots have a unique combinatorial histone code, not found at other functional elements, potentially recruiting specialized epigenetic readers. Using F1 hybrid mice we find that this hotspot epigenetic state and chromatin accessibility is assembled on the homolog targeted for DSBs where PRDM9 binds, but not the homolog used for repair. We identify an ATP-dependent chromatin remodeling enzyme that interacts with PRDM9 and is necessary for PRDM9-dependent epigenetic modification and DNA accessibility at hotspots, but not promoters. Subsequently, in male mice lacking the remodeling factor, DSBs are retargeted to non-hotspot sites. This results in errors in both DNA repair and synapsis of homologous chromosomes leading to sterility, a phenotype similar to loss of *Prdm9*. Together these data provide a model for hotspot activation where PRDM9 functions as a specialized meiotic pioneer factor by binding hotspots sequences in closed chromatin, creating a unique epigenetic signature, and recruiting additional factors to open chromatin in preparation for proper placement and repair of DSBs.

O-16: Scarless repair of kidney injury in African Spiny mice (*Acomys cahirinus*)

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Scar-free wound repair with tissue regeneration are properties thought to be restricted to a few species of amphibians, teleosts, and lower vertebrates. Yet fetal tissues of all mammalian species examined to date exhibit scarless regeneration *in utero*. Most mammals lose the ability for regeneration around the time of birth and thereafter heal wounds by fibrosis, with consequent loss of organ function. An exception to this pathogenic sequence is found in the Old World clade of muroid rodents known as African spiny mice (genus: *Acomys*). To avoid predation, *Acomys* species evolved a scarless, regenerative wound healing response to traumatic dermal injury. However, whether scar-free wound repair in *Acomys* extends beyond skin to vital internal organs is not known. We tested this by using two aggressive kidney injury models known to produce severe renal fibrosis in mice. Remarkably, despite equivalent acute kidney injury, there was rapid restoration of nephron structure and kidney function without fibrosis in *Acomys,* compared to extensive fibrosis leading to renal failure in *Mus musculus*. These results suggest *Acomys* species have evolved genomic adaptations for wound healing that activate regenerative repair pathways not only in skin, but also in vital internal organs. A preliminary analysis of gene expression reveals a marked difference in the activation of a transcriptional response in acutely injured mouse kidneys compared to those from *Acomys*. Our findings have important implications for discovering a long-sought evolutionary solution to internal organ injury and regeneration.

Mary Lyon Awardee Lecture

O-17: Evolution and function of the mammalian pseudoautosomal region

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The production of haploid gametes during meiosis is dependent on the homology-driven processes of pairing, synapsis, and recombination. On the mammalian heterogametic sex chromosomes, these key meiotic activities are confined to the pseudoautosomal region (PAR), a short region of near-perfect sequence homology between the X and Y Chromosomes. Paradoxically, despite its established importance for meiosis, the PAR is one of the most rapidly evolving loci in the mammalian genome. The mechanisms that drive the instability of this genomic region and safeguard correct X/Y segregation in the wake of frequent homology-disrupting mutations are poorly understood. To address these gaps in our knowledge of PAR biology, my lab is using a combination of cytogenetic methods, innovative sequencing pipelines, and novel bioinformatic analyses in multiple rodent model systems. Prior work and on-going experiments aim to address four key questions: (1) What is the scope of PAR diversity within species and between closely related species? (2) What are the consequences of PAR divergence for meiosis and fertility? (3) How does the meiotic function of the PAR constrain sex chromosome evolution? (4) What epigenetic and molecular features bestow the PAR with its unique biological properties? Collectively, these investigations are providing critical insights into PAR function and evolution, highlighting this enigmatic locus as a model of genome instability, and spearheading novel experimental approaches that can ultimately be extended to study other complex genomic regions.

O-18: Dissecting the mechanistic basis of the Dobzhansky–Muller incompatibility causing F1 hybrid sterility in *Mus musculus*

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Hybrid sterility (HS) in hybrids between diverged populations is a major contributor to speciation. The Dobzhansky–Muller (DM) model, a widely accepted explanation for HS, proposes epistatic incompatibilities involving two or more genes between two species. Male HS involving two subspecies of the house mouse, *M. m. musculus* (PWD/Ph) and *M. m. domesticus* (C57BL/6J), is a model for study of HS. This F1 hybrid model was used to map the only known mammalian speciation gene, *Prdm9 (Hst1)*, which encodes a histone methyltransferase that activates and determines locations of recombination hotspots in mice and other mammals. Deficiency of *Prdm9* results in sterility in both sexes. Genetic analysis has shown that epistatic interaction between the $Prdm9^{B6}$ allele with the $Hstx2^{PWD}$ locus on the PWD X Chromosome contributes to HS in (PWD x B6) F1 hybrid males, but the mechanism is unknown. In this study, we show that hemizygosity for the $Prdm9^{B6}$ allele in B6.PWD-Chr.X.2 congenic male mice (genetically B6 except for ~50Mbp of the PWD X Chromosome including the $Hstx2^{PWD}$ locus) leads to meiotic arrest with unrepaired DSBs and chromosomal asynapsis, an HS phenotype similar to that of (PWD x B6)F1 hybrid males. We show that hemizygosity of the $Prdm9^{B6}$ allele and presence of ~50 Mbp of the PWD X Chromosome are necessary and sufficient for these phenotypes in an otherwise B6 genetic background. In contrast, mice hemizygous for the $Prdm9^{CAST}$ or the $Prdm9^{PWD}$ allele in the B6.PWD-Chr.X.2 background are fertile. Genomic sequence data provided evidence for a repetitive DNA in the PWD X Chromosome, which could sequester $PRDM9^{B6}$ via many iterations of the allele-specific binding sites. This could lead to inefficient hotspot activation on the autosomes. Therefore we propose the cause of HS in this model is not hotspot asymmetry, but insufficiency of PRDM9-mediated hotspot activation.

O-19: Gene x environment interactions cause sperm decline and male infertility in mice

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Early-life exposure to environmental chemicals has been proposed as a potential cause of sperm decline in men, but this hypothesis is difficult to study directly. Sperm count varies due to genetics, as well as myriad lifestyle and environmental factors that affect reproductive health. Some men may be more susceptible than others to specific exposures. This issue is exacerbated by the fact that infertility can be caused by early life exposures with effects that only manifest years later, a paradigm known as the developmental origins of adult diseases (DOHaD). Estrogenic chemicals are leading candidates for specific toxicants contributing to sperm count decline. We use the Collaborative Cross (CC) mouse population-based model to understand how individual genetics interacts with estrogenic chemicals to alter male reproductive tract development, to decrease sperm count, and to cause male infertility. A screen of adult male mice belonging to 30 different strains shows that neonatal exposure to the synthetic estrogen diethylstilbestrol (DES) results in testicular degeneration and near complete sperm loss in several CC mouse strains but not in others. Reproductive phenotype distributions suggest a polygenic architecture of susceptibility. DES exposure altered male reproductive development in sensitive mouse strains as young as 14 days of age, and these changes led to adult infertility. Critical early events include lack of Leydig cell proliferation, delayed Sertoli cell maturation, and subsequent germ cell loss. Our results point toward the genetic mechanisms of susceptibility and provide new insights on the relative contribution of three distinct components of disease etiology – environmental effects, genetic effects, and gene x environment interactions (GxE).

O-20: Deficient LRRC8A-dependent Volume-Regulated Anion Channel (VRAC) activity is associated with male infertility in mice

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We previously described ébouriffé (*ebo*), a spontaneous mouse mutation associated with infertility, and identified *ebo* as a 2-bp frameshift deletion leading to a premature stop codon of the *Lrrc8a* gene. The truncated protein (c.1325delTG, p.F443*) is missing leucine-rich repeat domains LRR3 to LRR 17, but preserves the transmembrane domains. Ion channel-controlled cell volume regulation is of fundamental significance to the physiological function of sperm. In addition to volume regulation, LRRC8A-dependent Volume-Regulated Anion Channel (VRAC) activity is involved in cell cycle progression, insulin signaling, and cisplatin resistance. Nevertheless, the contribution of LRRC8A and its dependent VRAC activity in the germ cell lineage remain unknown. By utilizing the $Lrrc8a^{ebo}$ mutation and genetically-engineered mouse models, we demonstrate that LRRC8A-dependent VRAC activity is essential for male germ cell development and fertility. *Lrrc8a*-null male germ cells undergo progressive degeneration independent of the apoptotic pathway during postnatal testicular development. *Lrrc8a*-deficient mouse sperm exhibit Multiple Morphological Abnormalities of the Flagella (MMAF), a feature commonly observed in the sperm of infertile human patients.

O-21: Targeting the MYC G-Quadruplex Induces Cancer Cell Death

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The transcription factor MYC plays a major role in cancer initiation, progression, and maintenance in many cancers, including multiple myeloma. However, it has proven difficult to develop small molecule inhibitors of MYC. One potentially attractive route to pharmacological inhibition of MYC has been the prevention of its expression through small molecule-mediated stabilization of the G-quadruplex (G4) present in its promoter. G-quadruplexes (G4s) are noncanonical DNA structures that regulate transcription and often occur in the promoter regions of many genes. The regulatory role of G4s makes them attractive for targeted intervention, however, small ligands capable of discriminating between different G4 structures are rare. We have identified a small molecule, D089 and additional analogs, that preferentially downregulates MYC transcription by a G4-dependent mechanism, compared to other G4-containing oncogenes, in human multiple myeloma cells. This downregulation, which is mediated by stabilization of the MYC G4, leads to cell cycle arrest and cell death of the myeloma cells by a caspase 3-independent mechanism. We have found a number of other cancers within the NCI-60 panel of cancer cell lines to be susceptible to inhibition by this G4 stabilizer. Gene expression analysis and qPCR experiments has demonstrated that MYC and several MYC target genes were downregulated upon treatment with this compound, while the expression of several other G4-driven genes was not affected. Furthermore, we have solved the structure of the MYC G4 in complex with the analog DC-34 by using NMR spectroscopy and illustrate specific contacts responsible for their interaction.

O-22: The pluripotency regulator *Prdm14* hijacks hematopoietic regulatory *Cbfa2t3* to initiate leukemia in mice

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Despite advances in chemotherapeutics that improve survival rates in leukemia patients, most adults and relapsed patients succumb to the disease. Poor outcomes are associated with the presence of cancer initiating cells with stem cell phenotypes that initiate disease and sustain long-term tumour growth. Our lab identified *Prdm14* as a potent lymphoid leukemia oncogene. *Prdm14* is a pluripotency regulator central to embryonic stem cell identity and primordial germ cell specification. Numerous studies have linked *PRDM14* to human cancer, yet how amplification or overexpression of *PRDM14* contributes to oncogenesis remains unclear.

Using a ROSA26-FLOX-STOP-PRDM14 inducible mouse model, we show that *Prdm14* expression in hematopoietic stem cells leads to progenitor cell deregulation followed by rapid onset and fully penetrant NOTCH1-driven T-cell acute lymphoblastic leukemia (T-ALL). To gain mechanistic insight into how *Prdm14* initiates cancer, we are exploiting our model to study its interacting partners prior to leukemia onset. *In vivo* affinity purification/mass spectrometry in pre-leukemia and leukemia tissues identified CBFA2T3 as a major PRDM14-interacting partner that may explain progenitor expansion and lineage skewing. Candidates were confirmed and interacting protein domains were determined using tagged deletion constructs, providing clues about the interaction's functional consequences. To determine the requirement for CBFA2T3 in leukemogenesis, we placed our PRDM14-leukemia model on *Cbfa2t3* genetically deficient backgrounds. Mice deficient in *Cbfa2t3* do not develop signs of disease after 150 days, whereas *Cbfa2t3* heterozygotes develop T-ALL of the same cell type as the original model but with longer latency.

Together our data suggests that PRDM14 hijacks proteins present in hematopoietic progenitors to cause ALL, as removing its key protein partner prevents leukemia. Consistently, PRDM14 does not cause leukemia when expressed in mature hematopoietic cells. These experiments will determine how *Prdm14* expands progenitor cells and initiates cancer, informing its role in development and malignancies.

O-23: The use of an untargeted metabolomics approach to determine the functional consequences of differences in host genetics and the gut microbiota in *Apc*^{Min}/+ intestinal tumorigenesis

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Colorectal cancer develops due to the accumulation of genetic mutations, often including loss of the tumor suppressor adenomatous polyposis coli *(APC)* gene. Environmental factors such as the gut microbiota (GM) also play a role in tumor development. We observed significant differences in intestinal tumor multiplicity between two *Apc*^{Min} mouse colonies; C57BL/6J-*Apc*^{Min} (B6-*Min*/J) from The Jackson Laboratory (JAX), and C57BL/6JD-*Apc*^{Min} (B6-*Min*/D) from the University of Wisconsin. To determine the underlying contributors to this phenotypic variability, we used complex microbiota targeted rederivation to rederive embryos of the two *Apc* mutant colonies using surrogate dams harboring complex GMs from two different sources (GM1 originally from JAX or GM2 originally from Envigo), generating four *Apc*^{Min} groups. We found that all four groups had distinct tumor counts, with rederivation of *Min*/J embryos with GM2 partially restoring the original *Min/D* phenotype of the UW colony, and GM1 partially suppressing the *Min/D* phenotype. Thus, host genetic divergence between *Min*/J and *Min*/D, and the GM both contributed to the initially observed differential tumor phenotype. Whole-genome deep sequencing was performed using host tissue for characterization of divergence between the two substrains, and the GM composition of GM1 and GM2 was determined via next-generation sequencing of the microbial 16S rRNA gene in fecal and small intestinal tissue samples. To determine functional differences as a result of genomic and microbiota community differences, we employed untargeted metabolomics using liquid chromatography coupled with mass spectrometry. Genomic analysis predicted changes in bile acid (BA) metabolism due to a genetic variant in fatty acid binding protein 6 (*Fabp6*). This variant correlated with differential gene expression levels between *Min*/J and *Min*/D, and with differential abundances of BA intermediates determined via metabolomics analysis. Thus, integrated Omics analysis suggests an important role for BA metabolism in tumorigenesis, and demonstrates how host-environment interactions can shape a given disease phenotype.

O-24: Dynamic chromatin underlies intestinal stem cell differentiation

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Changes to chromatin state are a key mediator of gene expression programs and underlie differences in cell fate in many tissues. However, it remains unclear whether changes in gene regulation in different cell types merely reflects the activity of transcription or whether a dynamic and permissive chromatin environment primes a cell for these changes. By examining the global landscape of chromatin in multiple differentiated and stem cell types we can uncover novel regulatory networks and determine whether changes in differentiation state are driven by chromatin state changes. Using a Sox9EGFP reporter mouse (STOCK Tg(Sox9-EGFP)EB209Gsat/Mmucd) we investigated the dynamics of the native chromatin state in the mouse intestine. Within the intestinal crypt, levels of *Sox9* expression defines multiple differentiated cell types as well as a pool of stem cells necessary for proper homeostasis. We performed an integrated analysis of expression, open chromatin, and 5-hydroxymethylcytosine (5hmc) to define the regulatory landscape in these populations of cells. We found that chromatin state is highly dynamic, where both 5hmc and open chromatin correlate with changes in gene expression. Although many promoters are constitutively open in these cell types, tens-of-thousands of intronic regions are unique to a specific population. Profiling of transcription factor motif usage and expression suggests key positive and negative regulatory networks critical for defining stem and differentiated cells. We are validating these predictions by modulating chromatin state and monitoring the gene expression and cell fate changes in organoid models. This study has led to new insight into the dynamic chromatin changes underlying specification of differentiated and stem cell populations in the mouse intestine.

O-25: Determining the mechanism for lung lipid defects in a *Mecp2* mutant mouse model for Rett syndrome

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder of females primarily caused by mutations in the X-linked gene, methyl-CpG binding protein 2 (*MECP2*). RTT patients experience debilitating verbal and motor defects, metabolic disturbances, and severe respiratory distress.

MECP2 regulates gene transcription by anchoring a co-repressor complex containing histone deacetylase 3 (HDAC3) to DNA. MECP2 deficiency diminishes binding of this complex, resulting in profound lipid abnormalities in the brain and liver of *Mecp2*-mutant mice. Interestingly, the lung, an uncharacterized tissue in RTT, also participates in *de novo* lipogenesis.

We show for the first time, a striking accumulation of lipids in the lungs of *Mecp2* mutant mice. MECP2 is highly expressed in pulmonary lipid-producing alveolar type 2 (AE2) cells. To test the presence of a local lipid metabolism defect, we deleted *Mecp2* from AE2 cells in the lung; astonishingly, these mice exhibit elevated lung lipids, perturbed baseline breathing, and a heightened response to respiratory challenges, indicating some respiratory features of RTT have a pulmonary origin. Parallel studies in mice with a neuron-specific deletion of *Mecp2* indicate that loss of *Mecp2* in hindbrain causes distinct respiratory symptoms. These studies offer therapeutic hope; remarkably, statin drug administration reduces lung lipids and improves respiratory symptoms in *Mecp2* mutant mice.

Interestingly, MECP2 binds to the HDAC3-containing co-repressor complex in the lung. To identify lipid synthesis targets of this complex, we are performing single-cell RNA sequencing on wildtype and *Mecp2* mutant lipid-producing AE2 cells. Subsequently, ChIP-qPCR will allow us to determine if gene misregulation is caused by loss of MECP2-HDAC3 binding. Importantly, our findings will provide a better understanding of MECP2's role as a transcriptional regulator in non-neuronal tissue and support the repurposing of therapeutics in hopes of rescuing a core symptom of RTT.

O-26: Standardized characterization of mouse models for genetic diseases with intellectual disabilities unravelled unique behavioural profiles

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Major progresses have been made elucidating new genes involved in intellectual disability (ID). Now we need to elucidate molecular mechanisms underlying cognitive deficits to go further for therapies. Nevertheless we need robust mouse models carrying mutations of genes involved in ID, with consistent behavioural and neurological phenotypes. In addition the observation of genes contributing to similar class of ID in human would be reinforced if another animal model will present with similar cognitive defects.

Thus we decided to generate and characterize 40 mutant lines for ID genes, that were selected based on the frequency of mutations, with several of those corresponding to *de novo* mutations. Standardized behavioural screen were carried out, including several tests allowing to evaluate a wide range of functions or their pathologies, neurological reflexes and specific motor abilities, open field exploration and anxiety-related behaviour, sensorimotor gating or learning and memory processes for cognition. In some lines additional tests were performed to further characterize abnormalities observed or to extend phenotypic traits related to the individual genes. These tests allowed us to explore several research domains criterias including arousal, regulatory, cognition systems with negative valence.

Then a classification of mutated genes was drawn based on phenotypic traits. These data show heterogeneity of phenotypes between mutation types, recapitulating some of the human features, and suggesting thus the relevance of these mutants for better understanding human syndromes. Nevertheless, three main classes were identified based on activity phenotypes, to which other motor abilities or cognitive deficits were associated. Some of these mutants underwent pharmacological treatments to potentially reverse behavioural changes, and to screen for potential therapeutics.

A hitmap of behavioural phenotypes is presented, together with selected mutant results, emphasizing the importance of such programs for discovery/deciphering of genetic etiological causes of mental retardation and autism, and to build appropriate therapeutic strategies.

O-27: Mutagenesis with real-time assignment of cause and effect

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Forward genetics once required genetic mapping; then physical mapping to determine the gene content of the critical region; then the search for a discrete causative mutation. We have automated this process, and routinely identify mutations responsible for even subtle quantitative phenotypes at the same time those phenotypes are detected in screening. Whole exome sequencing is performed on G1 mutant males to identify all the mutations they may transmit to their G2 and G3 descendants. G2 and G3 mice are genotyped at all mutation sites to determining zygosity at each mutation site. G3 mice are each screened for >80 phenotypes. To date, 2,720 pedigrees incorporating 88,140 G3 mice and 148,782 mutations in 2,300 genes have been phenotypically assessed. Nearly 13 million tests of the null hypothesis (non-association of mutations with phenotypes) have been performed. A total of 1,477 mutations in 1,048 genes have been assigned putative responsibility for a total of 7,628 phenotypic readouts. Our focus is predominantly in the area of immunity, where 991 mutations in 728 genes have been assigned responsibility for 6,274 phenotypes. However, many mutations causing metabolic, neurobehavioral, and developmental disorders have been identified as well. Our best estimate of saturation suggests 33% of all genes have been severely damaged or destroyed with variant alleles examined twice or more in the homozygous state for phenotypic effects. While about 1/3 of all genes are essential and therefore not easily assessed in knockout mice, viable hypomorphic alleles of these genes often show striking phenotypic effects in the homozygous state. The speed and economy of studying ~60 mutations per pedigree in many independent screens exceeds that of the gene targeting approach. A bank of point mutations is progressively developed, with >400,000 incidental mutations so far collected and made available for community use. More than 624 phenotypic mutations are currently displayed and described in detail at **https://mutagenetix.utsouthwestern.edu**

O-28: Role for genetic variation in the NLRP1 inflammasome locus in Influenza A Virus pathogenesis

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Influenza A Virus (IAV) is an enormous public health burden, leading to high morbidity and mortality across the globe. Vaccines for IAV are available but often ineffective; many individuals fail to mount a protective antibody response, leaving them at risk for severe disease. Human studies have shown that host genetic factors play a significant role in regulating the antibody response to infection and vaccination. However, these studies have been severely limited by inability to control important variables such as dose and prior exposure. We have used the Collaborative Cross (CC), a genetically diverse and experimentally tractable mouse genetics reference population, to study the host genetic factors that regulate antibody response to IAV. We infected 110 CC-F1 lines with the pandemic 2009 H1N1 IAV and measured the quantity of IAV-specific antibody at multiple timepoints post-infection. Antibody responses were highly variable across lines for different subtypes and timepoints, with heritability estimates ranging from 5-80%. We utilized this heritable variation to map the quantitative trait loci (QTL) underlying the magnitude of antibody response. One of the strongest QTL identified underlies variation in magnitude of IgG3 at day 10 post-infection and encompasses a 2Mb region on Chromosome 11. Based on haplotype effects as well as prioritization of genes with polymorphisms likely to have functional consequences, we identified variants in the NLRP1 inflammasome locus as the strongest candidates driving the QTL. We have subsequently shown than *Nlrp1b* knockout mice are more resistant to infection than littermate controls and mount stronger responses for all antibody subtypes, and are further probing the mechanism through which NLPR1 is activated by IAV and contributes to disease and antibody response. Our results suggest a novel role for the NLRP1 inflammasome in IAV pathogenesis and illustrate the utility of the CC for studying IAV pathogenesis and immunity.

O-29: Mapping of susceptibility alleles for Ebola hemorrhagic fever pathogenesis and resistance

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Ebola virus (EBOV) causes severe hemorrhagic fever in humans and nonhuman primates with high fatality and a major public health concern in Africa. A recent study utilizing a genetically diverse panel of recombinant inbred mice (Collaborative Cross mice, CC) revealed that host genetic diversity plays a major role in EBOV pathogenesis and resistance. To understand the host contribution to disease, a panel of 8 CC-strains were infected intraperitoneally with 100 PFU of mouse-adapted (MA-)EBOV. A range of disease phenotypes were observed, from mild clinical disease to severe Ebola hemorrhagic fever (EHF)-associated pathology, and/or death. Two strains, CC074/Unc and CC011/Unc, were representative of the extreme phenotypic outcomes that can occur after infection. CC074/Unc presented with a unique, susceptible response driving EHF pathology and the high mortality phenotype by day 5-6, whereas CC011/Unc presented with a highly resistance outcome characterized by minimal clinical disease development and full recovery by day 11 post infection. All resistant and susceptible animals had high virus titers in the blood at day 6. We generated an F2 population to understand the genetic factors that regulated divergent EBOV outcomes and to map specific susceptibility alleles for EBOV weight loss, disease development, pathology, mortality, and virus titer. We infected 298 animals, approximately equal gender distribution, with MA-EBOV and monitored for 6 days. Susceptible F2 mice presented with high weight loss and mortality and many displayed pathological findings consistent with EHF, including internal hemorrhage, splenomegaly, and hepatic discoloration. In contrast, resistant F2 mice displayed minimal weight loss and minimal liver/spleen pathology. All animals tested had evidence of virus replication. The data are being used to genetically map, identify, and characterize susceptibility alleles that specifically regulate EBOV replication, pathogenesis, the development of lethal EHF and will provide insight into the molecular-genetic mechanisms regulating the pathogenesis of EBOV, potentially informing development of new therapeutics and vaccine strategies.

O-30: Interactions between the host genome and the gut microbiome determine susceptibility to CNS autoimmune disease

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Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS), which represents the leading cause of non-traumatic neurological disability in young adults. Like most common chronic diseases, MS is multifactorial and polygenic. Approximately 30% of MS risk is genetically determined, while 70% is attributed to the environment or gene-environment interactions. Recent studies have demonstrated that the gut microbiome in MS exhibits significant alterations, including depletion of the *Lactobacillus* genus. Animal model studies have suggested a causal role for gut dysbiosis in MS, although the underlying mechanisms remain unclear.

Here, we leverage natural genetic variation in the mouse to identify interactions between the host genome and the gut microbiome that determine susceptibility to disease in a model of MS, experimental autoimmune encephalomyelitis (EAE). In order to better approximate the genetic diversity present in human populations, we utilized wild-derived PWD/PhJ mice, which are greatly divergent from conventional laboratory strains, such as C57BL/6J. Additionally, a panel of 27 consomic strains carrying single PWD/PhJ chromosomes on the C57BL/6J background was used. We show that PWD-derived loci exhibit potent, bidirectional, and sex-specific effects on EAE severity. Using 16S rRNA DNA sequencing, we characterized the gut microbiome in the consomic and the parental strains, and identified distinct strain-specific profiles. Specific taxonomic units associated with differential susceptibility to EAE were identified. Additionally, gut microbiome manipulation was performed using co-housing or microbiome transplantation to germ-free mice, which demonstrated direct host genotype-dependent effects of the microbiome on EAE susceptibility. 16S and qPCR analyses identified two *Lactobacillus* species that were inversely correlated with EAE outcomes across the two different experimental approaches. These studies reveal the presence of complex bidirectional interactions between the host genome and the gut microbiome that determine susceptibility to CNS autoimmunity, and identify specific microbiome constituents that could be targeted therapeutically or prophylactically.

O-31: Using a reduced complexity cross to identify host susceptibility factors to SARS-CoV infection

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Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) was identified in 2002 as the etiologic agent of a global outbreak of atypical pneumonia and acute respiratory distress syndrome. The host factors contributing to either survival or progression to severe disease remain unknown. To address this question, we used a systems genetics approach driven by the serendipitous observation that Balb/cJ mice are resistant to infection with SARS-CoV while Balb/cByJ mice are extremely susceptible. Taking advantage of recent genotyping advances and the close genetic relationship between these substrains, we designed an F2 cross between the resistant Balb/cJ and susceptible Balb/cByJ to enable mapping of host alleles guiding the response to SARS-CoV infection. 266 mice were infected with SARS-CoV and monitored during a four day infection. We observed that susceptibility to SARS-CoV infection was dominant and that weight loss and pulmonary hemorrhage were significantly correlated ($p<0.001$) while weight loss and pulmonary titer were not correlated ($p=0.5608$). We identified a single quantitative trait locus of major effect on Chromosome 15 that effected day four weight loss. After mapping with existing SNP markers this QTL covers a 48Mb region that includes 189 novel variants between Balb/cJ and Balb/cByJ in 103 genes or intergenic regions. Testing additional Balb/c substrains reduced the candidate genes by over 75% and currently we are narrowing the QTL interval using PCR based fine mapping. Given the small number of variants we anticipate identifying both the gene and the causative mutation that dictate the varying Balb/c responses to SARS-CoV infection. Analysis of the early response to infection in susceptible and resistant Balb/c substrains suggests that aberrant immune signaling may be responsible for the differential infection outcome. In addition to improving our understanding of SARS-CoV pathogenesis, this work highlights the care that must be taken in comparing phenotypes between mouse substrains.

O-32: Of mice and men – the host response to influenza virus infection

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Every year, influenza virus (IV) infections cause about 50,000 deaths in the USA. Controlled experimental studies in the mouse infection model allow us to obtain a better understanding of the biological, genetic and environmental factors that contribute to severe IV disease in humans. However, mouse-human cross-species comparisons are often compromised by the fact that animal studies concentrate on infected lungs whereas almost all human studies analyze peripheral blood. In addition, current IV studies in human patients do not consider genetic background as contributing factor to molecular and clinical responses or susceptibility. To address these limitations, we performed a cross-species comparison of transcriptome changes after IV infection in the peripheral blood from human patients and blood from mice of the genetically highly diverse Collaborative Cross strains. We showed that responses to IV infections are strongly influenced by genetic background. Furthermore, changes in gene expression are very similar in mice and humans. Thus, the mouse represents a highly valuable *in vivo* model system to discover and validate genes that may be important players in the human host.

O-33: Genetic Regulation of Airway Mucins and Consequences on Host Defense in Mice

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Background

Mucus secretion and mucociliary clearance in the airways represent major components of host defense against respiratory pathogens and other noxious agents. Aberrant mucus levels are also associated with multiple obstructive airway diseases such as asthma, COPD, and cystic fibrosis. We sought to identify novel regulators of the two major mucins proteins in airway mucus, MUC5AC and MUC5B, and assess the contributions of novel regulators on host defense.

Methods

We applied a house dust mite allergen challenge model of asthma to incipient lines (n=154) of the Collaborative Cross (CC). We collected bronchoalveolar lavage (BAL) samples 72 hours after allergen challenge and quantified MUC5AC and MUC5B by agarose gel electrophoresis followed by Western blotting. Each CC mouse genotyped and QTL mapping was performed using BAGPIPE software.

Results

Incipient CC lines exhibited a broad range of MUC5AC and MUC5B in BAL. QTL mapping identified distinct, trans protein QTL loci on Chr 13 for MUC5AC (at 75 Mb) and Chr 2 for MUC5B (at 154 Mb), respectively. Subsequent analysis of the MUC5B QTL allele effects allowed us to narrow the Chr 2 QTL region to a ~2 Mb region, and we identified *Bpifb1* (BPI fold containing family B, member 1) as a candidate gene. We show that BPIFB1 is expressed in the large airways and colocalizes with MUC5B, and that its expression parallels *Muc5b* mRNA and protein levels after allergen challenge. *Bpifb1* knockout mice expressed higher levels of MUC5B at baseline and after allergen challenge, decreased mucociliary clearance, and decreased clearance of respiratory pathogens such as *Streptococcus pneumoniae*.

Conclusions

Our results indicate that the concentrations of MUC5AC and MUC5B in BAL after allergen sensitization and challenge are controlled by distinct, trans-acting genetic loci. Variation in *Bpifb1* is causally related to MUC5B levels and mucociliary clearance, and consequently, response to respiratory pathogens.

O-34: Epigenetic control of complex disease susceptibility

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Complex trait diseases afflict >2 billion people worldwide. The rapid rise in particular of early life disease carries long-term health burden including heart disease, diabetes and stroke, making the issue one of the world's chief economic and health care challenges of the day. While numerous studies have established a genetic framework for understanding metabolic disease, the contribution of critical regulatory layers, in particular epigenetic regulation, remain poorly understood. Our focus couples epigenomic analysis with functional genetics in model organisms to understand chromatin-coupled disease events and their direct implications for disease etiology. Our focus has been to understand non-genetic disease heterogeneity and thus understand the spectrum of disease potential that lies within each individual. These efforts have uncovered novel roles for Polycomb silencing in buffering beta-cell dedifferentiation, as well as signalling modules that drive and potentiate browning of adipose tissues. They have revealed mechanistic underpinnings for intergenerational control of non-genetic variation and what we believe to be the first stochastic disease 'switch' yielding distinct phenotypic 'on' and 'off' states in mouse and potentially man. The data suggest a highly regulated landscape of phenotypic variation defines mammalian disease.

O-35: Using Advanced Mouse Populations to Probe Genes and Networks that Drive Addiction-Related Behavior

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Substance use disorders (SUDs) are a public health concern with significant consequences for affected individuals and society. Genetics contribute significantly to SUDs in humans, but very few risk genes have been identified. Gene finding efforts have been hampered by genetic complexity, environmental factors that are largely unknown and insufficient power in existing experimental cohorts. Mice have been used as an experimental system to identify genes implicated in addiction-related behaviors, leading to the discovery of pathways that are relevant in human SUDs.

We identified two Collaborative Cross (CC) strains, CC004 and CC041 that differ strikingly in locomotor response to cocaine and responding for cocaine in a self-administration protocol. Cocaine pharmacokinetics and dopamine dynamics do not differ between the strains. However, norepinephrine levels are increased in the nucleus accumbens of the high responding strain and serotonin is decreased in the dorsal striatum of the low responding strain.

We conducted mapping in an F2 generated by crossing the low responding CC strain to C57BL/6NJ. We identified three significant quantitative trait loci on Chromosomes 7, 11 and 14. The Chr 7 QTL fit the expected allelic differences based on parental phenotypes. CC founder strains, NOD/ShiLtJ and NZO/HlLtJ, defined the haplotype at the Chr 7 QTL, indicating that the one of these strains harbors the causal allele. Bioinformatic strategies and RNA-seq data from an independent Diversity Outbred (DO) population were used to identify *Chst8* as a high priority candidate.

The genetic and phenotypic diversity of advanced mouse populations like the CC can be exploited to identify extreme phenotypes. Rapidly developing genomic and bioinformatic resources for the CC and DO allow for systems genetic approaches to identify genes and gene networks that drive addiction-related processes.

O-36: Exploiting dynamic open chromatin in mouse dopamine neurons reveals Parkinson-associated variation in an *SNCA* enhancer: a paradigm for illuminating functional noncoding variation

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A significant challenge in human genetics lies in illuminating the cellular context(s) in which indicted noncoding variants mediate their effect. Exploring where and when noncoding variants modulate their cognate genes requires systematic acquisition and application of biological insight.

Given the preferential degradation of midbrain (MB) dopaminergic (DA) neurons in Parkinson disease (PD), we prioritize this as a context in which PD-associated variation likely acts. Thus, we set out to capture chromatin accessibility data from *ex vivo* populations of MB DA neurons, sorting DA neurons from Tg(Th-EGFP)DJ76Gsat BAC transgenic mice at embryonic and postnatal timepoints of wildtype mice and those harboring a familial PD mutation in α-synuclein (*SNCA*).

Doing so, we have assembled catalogs of putative regulatory elements to investigate noncoding variation contributing to PD risk. In the catalog generated at embryonic day 15.5, our data capture the vast majority (77%) of previously validated MB enhancers in the VISTA browser. Transgenic analysis of six additional sequences in both zebrafish and mice confirms neuronal enhancer activity of all six.

One enhancer, at *SNCA,* directs reporter expression in catecholaminergic neurons of transgenic mice and zebrafish. Sequencing this enhancer (986 PD cases, 992 controls) revealed two tightly linked common variants (rs2737024, rs2583959) significantly associated with PD risk (OR=1.25; p=0.002). Genotyping a variant panel across *SNCA*, we identify 12 haplotypes; only one, containing the alternate alleles of the identified enhancer variants, is associated with PD (frequency in: PD=28.3%, controls=23.5%, p=0.004). Our work highlights the value of cell context-dependent guided searches for functional noncoding variation.

These catalogs are vast and much remains to be explored; an additional dozen disease associated variants with predicted effect on enhancer activity have been prioritized. We will report the results of this and the ongoing work designed to leverage the potential of the mouse to inform human genetic disease analyses.

O-37: Genetic control of physiological response to carbohydrate consumption

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The application of genomic approaches to understand unique responses to different diets has been understudied and remains poorly understood despite genomic applications to characterize complex disease. To advance precision dietetics, we are establishing a predictive measure for a major driver of obesity and metabolic syndrome: responsiveness to carbohydrate consumption. Our laboratory has demonstrated the effects of inter-individual response to diet in a study using four inbred mouse strains: C57BL/6J (B6), FVB/NJ (FVB), A/J, and NOD/ShiLtJ (NOD). Increased body fat gain and other negative health effects were observed in B6 mice consuming an American diet (high fat, high carbohydrate) and but not in B6 mice on a ketogenic diet (high fat, no carbohydrate). Negative health effects were not observed in FVB mice exposed to the same two diets. This suggested that the individual response to a high fat diet is more dependent upon the presence or absence of carbohydrates than exposure to the high fat diet alone for B6 mice, whereas FVB mice showed no differential responsive when carbohydrates were restricted. Analysis of the liver transcriptome in each strain shows up-regulation of 25 gene networks associated with adipogenesis in FVB mice when compared to B6 mice. An intercross (F2) population was generated to investigate responsiveness to carbohydrate restriction. Half of the F2 mice were placed on American diet and half on ketogenic diet for three months before characterizing changes to body composition and weight gain. Genetic analysis in each of the F2 populations revealed quantitative trait loci (QTL) on Chromosomes 4 and 5. We are systematically investigating genes within the QTL region and have developed a gene signature that appears to be predictive of response or non-response to carbohydrate restriction. This gene signature will be used to predict diet response in a genetically heterogeneous mouse population, the Collaborative Cross Recombinant F1 Hybrids.

O-38: Assessment of early-life lead exposure in a genetically heterogeneous mouse population

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Most human diseases result from a complex interplay of genetic, epigenetic and environmental factors, and current scientific approaches do not adequately capture these complex interactions. Intrinsic variability across the human population is associated with variable responses to environmental exposures, which can contribute to whether an individual is susceptible or resistant to a particular adverse outcome. Understanding such gene-environment interactions will enable a more accurate toxicant risk assessment and public health protection. Childhood exposure to lead, a potent neurotoxin for which there is no safe blood level, can impact many developmental and biological processes, most notably intelligence, behavior, and overall life achievement.To specifically address the influence of genetic background on adverse outcomes from lead exposure, we are utilizing a recombinant inbred intercross (RIX) mouse population which allows controlled exposures in a population setting to identify genetic polymorphisms that drive either susceptibility or resistance to lead toxicity and to elucidate the underlying molecular mechanisms. To this purpose, F1 male and female animals derived from crosses of Collaborative Cross inbred lines receive early life (birth through weaning) exposure to lead through lactation and then through drinking water (0, 0.1, or 0.5%) to mimic human exposure. Preliminary results reveal significant variation in blood lead levels, blood cell counts, body weight, and epigenetic modifications between strains exposed to the same dose. We are also assessing long-term effects of lead exposure on behavior and cognition in adult mice with early life exposure. Differences between strains could provide us the key in understanding mechanisms by which genetics, epigenetics, and toxic exposure contribute to neurological phenotype and provide new tools for exposure detection, risk assessment, and interventions to reduce lasting effects of early life exposure.

O-39: Generation and validation of increasingly complex alleles by genome editing

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Mouse models are valuable tools to understand genes functions, genetic diseases and to develop and test new therapeutic treatments *in vivo*. The ability to introduce tailored modifications within the mouse genome is essential to generate them. The CRISPR/Cas9 system has brought new perspectives for the generation of mouse models in a more efficient and precise fashion, at reduced price, all within a shorter time scale. We are developing protocols for the production of increasingly complex alleles. Alongside the generation of mutants, their validation represents a new challenge that is essential to meet to ensure research reproducibility. We will present our recent developments of processes for genome engineering. We will also show the first results of a new pilot for the use of the long-read sequencing for founder screening and model validation. With new processes for allele validation, we uncover further variability in the outcome of applying CRISPR/Cas9 to the modification of mouse early embryos. This includes discrete sequence changes, the generation of larger than expected deletions and chromosomal rearrangements. We will show how extensive validation recognises unwanted variants at early stages of the mutagenesis process and reduces the number of animals used for genome engineering.

O-40: Completion of first pass GENCODE gene annotation for the mouse reference genome

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The accurate identification and description of the genes in the human and mouse genomes is a fundamental requirement for high quality analysis of data informing genome biology and clinical genomics. Since 2012, the GENCODE consortium have been producing reference quality annotation of protein-coding genes, pseudogenes, long non-coding RNAs and small RNAs in mouse to provide this foundational resource. The GENCODE consortium includes both experimental and computational biology groups who generate primary data, create bioinformatic tools and provide analysis to support the work of expert manual gene annotators and automated gene annotation pipelines to improve and extend the Ensembl/GENCODE gene annotation. Manual annotation workflows use any and all publicly available data including Cap analysis gene expression (CAGE), RNAseq, SLRseq and PacBio long transcriptomic reads, along with the research literature to identify and characterise gene loci to the highest standard. Versioned GENCODE mouse gene sets are released approximately four times a year. Ensembl/GENCODE gene annotations are accessible via the Ensembl and UCSC genome browsers, the Ensembl FTP site, Ensembl Biomart, Ensembl Perl and REST APIs and gencodegenes.org.

GENCODE annotators have 'walked' across the entire reference mouse genome investigating the sequence, aligned data and computational predictions for each BAC clone in turn. The completed first pass annotation is anticipated to be released as GENCODE M20. Having completed the first pass annotation, GENCODE are also targeting specific loci for example to identify unannotated protein-coding and lncRNA genes and alternatively spliced transcripts and reassess legacy protein-coding gene annotation in the light of current data. As such, the numbers of both genes and transcripts is subject to change and while the existing human and mouse transcript annotations are of high quality, the abundance of evidence from new transcriptomic and proteomic datasets makes it absolutely clear that they are not yet complete.

O-41: Elucidating the putative regulatory role of GWAS-associated neural disease variants using STARR-Seq

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GWAS have indicted thousands of variants as contributing to an extensive catalog of phenotypes. LD further complicates this observation, yielding many variants with shared genetic evidence of involvement. The challenge of their functional dissection on a meaningful scale is demanding, requiring conceptual and technical innovation. Massively parallel reporter assays now enable high throughput functional assessment of thousands of variants simultaneously. While these assays improve our ability to identify functional variation, technical challenges remain; synthesized length restrictions (≤150 bp), haplotype of the DNA to be assayed, and the impact of promoter choice and cellular context.

We sought to overcome these challenges utilizing a haploid human cell line (eHAP1) as the source DNA to allow for capture of regions (300-400 bp) with specific alleles. We then employed massively parallel mutagenesis (PALS) to generate the alternative allele, overcoming both imprecise haplotype matching of capture and length restriction of synthesis.

To assess our methodology, we selected lead SNPs and SNPs in high LD ($r^2 \geq 0.8$), associated by GWAS with human neurological diseases. From this list, we captured, mutagenized, and assessed 3,027 SNPs for their effects on regulatory activity in SK-N-SH neural cells. All regions were separately cloned upstream of two different promoters: TATA (basal) and Syn1 (neuronal).

We identified 112 enhancers common to both promoters and additional enhancers specific to Syn1 (89) or TATA (74). Further, 27 SNPs elicit significant allele-dependent activity, from which we have selected ten for validation in luciferase and zebrafish transgenic reporter studies. To establish physical relationships between assayed SNPs and target genes, capture HiC experiments are also in progress. Finally, in a parallel effort to explore cell type dependent activities, we are actively repeating this experiment in a mouse *substantia nigra*-derived cell line. In this way, we can rapidly prioritize putative causal SNPs within GWAS regions for directed experimental validation.

O-42: Genetics of seizure sensitivity in the Collaborative Cross

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Epilepsy affects 3% of the human population and has significant morbidity and societal costs. Not all therapy strategies are effective as 30% of patients' seizures are refractory to existing drug treatments. There is a great need to tie together genetic variation and drug efficacy to identify treatment targets for refractory seizures. We are using the Collaborative Cross (CC) mouse population to study the genetics of seizure phenotypes with the ultimate goal of identifying novel seizure sensitivity genetic risk factors that are refractory to traditional anti-epileptics. The CC is an excellent resource because it contains a high level of genetic diversity that matches the human population, but exists as a panel of inbred lines that have repeatable phenotypes and genotypes. We hypothesize that seizure susceptibility in the CC population has a genetic component that can be mapped as a first step towards the identification of new candidate genes for seizure sensitivity. An initial screen of 19 strains has revealed a wide phenotypic variation for a number of seizure related traits, including myoclonic seizure (MS) threshold, generalized seizure (GS) threshold, flurothyl-induced kindling, and sudden unexpected death in epilepsy. In each case, one or more CC strains expanded the phenotypic range reported in classical mouse strains. Initially focusing on MS and GS thresholds, we were able to identify 8 QTL in a moderately sized intercross. These results support our hypothesis, provide strong evidence of feasibility, and demonstrate that interdisciplinary research can provide new insights with significant implications in a common human disease.

O-43: Exploring high-throughput sequencing data using Multi-string Burrows Wheeler Transforms

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In less than two decades, low-cost, High-Throughput Sequencing (HTS) technologies have revolutionized the study of genetics. The appeal of HTS is that, with a single assay, one can discern nearly all types of simple DNA polymorphisms (ex. SNPs, STRs, InDels), and structural variations. However, this wealth of genomic information comes with a significant downside-- the huge volume of unorganized data generated by HTS is nearly impenetrable for general inquiry. As a result, access to HTS data is usually indirect. That is, after it has been processed and organized by common bioinformatics pipelines, such as reference-based aligners or various types of de novo sequence assemblers.

In this talk I will describe an alternative method for gaining access to, and performing queries of, HTS data using a lossless compressed form of the raw sequenced reads, called the multi-string Burrows-Wheeler Transform (msBWT). And rather than going into the algorithmic details and performance advantages of the msBWT data structure, I will instead concentrate on how to use an msBWT to elucidate targeted questions of genetic interest. I will show examples of how we have used the msBWT to resolve a range of genomic and genetics questions including variant calling, CNV detection, local genomic assembly, tracking of transposable elements, comparative genomics, quantification of gene expression in RNAseq data, estimating allele specific gene expression as well as other questions.

O-44: The expanded BXD family : A cohort for experimental systems genetics and personalized precision medicine

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The BXD family of recombinant inbred strains (RIS) is an excellent resource to facilitate experimental systems genetics and personalized precision medicine. The size of the BXD family has been expanded two-fold, from 70 to 150 strains, creating an recombinant inbred panel well suited for modeling complex interactions among genetic variants, or across biomolecular phenotype levels. The BXD family is a reproducible resource, and allows for the detailed dissection of environmental factors that influence disease risk and treatment on a diverse genetic background.

All 150 BXD family members have been deeply sequenced at ~38X using Chromium linked-read technology, and have been deeply genotyped several times throughout there projection. This provides comprehensive genetic and physical maps to allow precise QTL mapping. Comparable to human cohorts, there are >5 million common and known sequence variants segregating within the BXD family. Approximately 10,000 recombinations between parental haplotypes have been located with a mean precision of ~100 kb. Based on empirical data and analysis of cis-eQTLs, it the BXD family provides a mapping precision of under ±2.0 Mb across most of the genome for low effect QTL. Mendelian loci (LOD>10) can be mapped with a precision of ±500 kb. The extended 150 strain BXD family is now sufficiently large to allow testing of epistatic interactions.

The BXD family is the most deeply phenotyped mouse family with ~ 6000 classical quantitative (clinical) phenotypes available obtained under various environmental conditions. Besides the BXD's well-structured phenome over 100 gene expression, proteome, metabolome, and metagenome data sets have been generated for many different tissues. The BXD family is an excellent resource to discover networks of causal and mechanistic relations among clinical phenotypes and millions of molecular and organismal traits, including metabolic syndrome, infection, addiction, and neurodegeneration, and longevity. Precisely matched cohorts can be raised under different conditions to study gene-by-environmental interactions, epigenetic modifications, replicability, and robustness of genome-to-phenome relations.

O-45: A suppressor screen in *Mecp2* mice implicates the DNA damage response in Rett Syndrome pathology

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The onset and expression of clinical features can vary widely in genetic diseases with a common molecular root, often due to second site gene modifiers. Identifying the modifiers represents a transformative discovery for the disease they alter, shifting understanding of pathogenesis and providing avenues for diagnosis, prognosis, and therapy development.

We carried out a forward genetic ENU suppressor screen in a mouse model for Rett syndrome (RTT), a neurological condition caused by mutations in methyl-CpG binding protein 2 (*MECP2*). MECP2 regulates key activities in the brain and body, with mutations impacting both adult and childhood neuropsychiatric and immune disorders. Whole exome sequencing of 92 mouse lines carrying modifiers that improve health traits and prolong life has identified genes that fall into a limited number of biological pathways. One of the earliest modifiers points to lipid metabolism as being perturbed in RTT, suggesting that metabolic modulation is a treatment avenue.

Here we show that mutations in multiple genes involved in the DNA damage response (DDR), which repairs double-stranded breaks (DSBs), can improve RTT-like symptoms in mice. Expression of DDR pathway components is perturbed in *Mecp2*-null brain and liver. In particular, H2AX, a marker of DSBs, is upregulated in the brain and liver of *Mecp2*-null mice. *In vitro* assays that measure the frequency of DSB repair pathway choice show that the rate of homologous recombination is elevated in *Mecp2*-null cells. Because neurons are non-dividing cells, an ongoing question is why mutations in this pathway improve symptoms.

Unbiased genetic screens can uncover unexpected pathways that contribute to disease pathophysiology and provide insight into therapies. Many of the lines carry more than one modifier locus, and combining modifiers from two different pathways greatly improves symptoms, suggesting that combination therapies will be effective in treating RTT.

O-46: *Uqcrh* KO mouse mimics mitochondrial respiratory chain disorder

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The ubiquinol:cytochrome c oxidoreductase hinge protein (UQCRH) is part of the assembly of the complex III (CIII) of the oxidative phosphorylation (OXPHOS) system. Mitochondrial respiratory chain disorders are the most common form of mitochondrial diseases. So far, a disease linked to *UQCRH* has not yet been described.

Under the aegis of the International Phenotyping Consortium (IMPC) the *Uqcrh* KO mouse was generated by deletion of exons 2 and 3. The phenotyping screen of the German Mouse Clinic examined all relevant organ systems of the mutant mice compared to controls. The deletion in these mice led to phenotypes with progressive functional impairment and premature early adult death. Analyses of blood parameters revealed e.g. elevated lactate and the development of hyperglycemia at young age. Enzymatic activity and protein expression of OXPHOS complexes were investigated in mouse tissue revealing a significant decrease in CIII activity in heart, brain and liver tissues. Whole exome sequencing and autozygosity mapping on two male first cousins from a consanguineous family with recurrent episodes of severe ketoacidosis, excess blood ammonia and hypoglycaemia and signs of encephalopathy revealed a homozygous *UQCRH* deletion of exons 2 and 3. Strikingly, several mouse phenotypes correlate with the clinical presentation of the patients at metabolic episodes. As observed in Uqcrh mouse tissue, cultured patients' primary fibroblasts showed incomplete assembly and decreased steady-state protein levels of CIII subunits and low CIII enzyme activity.

Here we describe the first viable homozygous complex III KO mouse model, which will give new insights not only in the human disease progression, but also in general mechanisms of the complex III assembly.

O-47: Discovery of new genes related to human metabolic disorders by high throughput mouse phenotyping

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Metabolic diseases such as obesity and type 2 diabetes mellitus cause increasing problems worldwide. To understand the relevance of the underlying genetic factors, large-scale research is required to investigate the role of so far unannotated mammalian genes that may show links to metabolic dysfunction in humans. The International Mouse Phenotyping Consortium (IMPC) is aiming to produce a knockout mouse line for every protein-coding gene. Highly standardized data related to various disease areas are generated by high-throughput phenotyping.

In an IMPC-wide analysis, we evaluated phenotypic data of more than 2,000 knockout strains covering glucose and energy homeostasis, body mass, and lipid metabolism. 974 gene knockouts caused strong metabolic phenotypes, 429 of those had not been linked to metabolism before. 51 genes were functionally unannotated in mice so far. We also searched for associations of unannotated genes to metabolic disease. In a query combining five GWAS, human orthologues of 23 unannotated mouse candidate genes were associated with metabolic disease in humans. To discover novel candidate genes, we used both literature-based evidence e.g. from KEGG as well as structural information on motifs in regulatory regions of genes (MORE sets) causing a strong metabolic phenotype to construct networks of interacting genes linked to metabolism. We could show that examples for both KEGG networks and MORE set-based networks largely overlap. Sharing regulatory elements also was a powerful tool to predict metabolic functions of so far unannotated genes. In general, with no other prior knowledge, the identification of shared MORE cassettes in known and novel genes opens up a new approach to unravel potential candidate genes, which are connected to a specific pathway or disease area. The results highlight the capacity of standardized systemic mouse phenotyping to identify human disease genes and to direct future in-depth research.

Reference: Rozman et al., 2018; Meehan et al., 2017

O-48: RGD's updated and redesigned Phenotypes and Models Portal: Data for precision models of human disease

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RGD (https://rgd.mcw.edu) has updated and redesigned the Phenotypes and Models Portal to simplify access to diverse data about models of human disease in rat and other mammalian species.

For the researcher interested in pursuing phenotype data, RGD's PhenoMiner tool provides a wealth of quantitative phenotype measurements for rat, as well as a new set of measurements for chinchilla, the model of choice for studying otitis media. Standardization of the information about the measurements made and the methods and conditions used gives users the ability to compare values within and across studies, strains, methods and conditions. In addition, RGD has performed statistical analyses of these data to provide "expected ranges" for a number of measurements in strains where a substantial body of data is available in PhenoMiner. Researchers can explore these data using the new Expected Ranges tool in the Phenotypes and Models Portal.

For a researcher looking for a model of a human disease the Phenotypes and Models Portal gives multiple entry points by which to narrow their selection. Search options, including a keyword search and searching by phenotype or disease, are available for the researcher interested in finding a particular rat strain. In addition, RGD curators have compiled lists of established rat models for a variety of human diseases as well as lists of genetic models and genome-edited rat strains for researchers interested in exploring the effects of alterations to particular genes. Information is provided about strain availability, about strain phylogenetics, and about establishing and maintaining a rat colony, as well as protocols for physiological and molecular methods. Additional links to information about non-rat disease models and to RGD's Disease Portal, as well as to a wide variety of resources outside RGD further expand the utility of RGD's redesigned Phenotypes and Models Portal.

O-49: Antipsychotic behavioral phenotypes of Collaborative Cross recombinant inbred inter-crosses (RIX)

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Schizophrenia is an idiopathic disorder that affects approximately 1% of the population, and presents with persistent delusions, hallucinations and disorganized behaviors. Antipsychotics are the standard treatment of schizophrenia, but are frequently discontinued by patients due to inefficacy and/or intolerable side effects. Chronic treatment with the typical antipsychotic haloperidol causes tardive dyskinesia (TD), involuntary and often permanent orofacial movements, in ~30% of patients. There is substantial inter-individual variation in liability to these adverse drug reactions (ADRs) and direct and indirect evidence suggest a role for genetic variation. Currently, there are no compelling algorithms to predict ADRs or efficacy in the treatment of schizophrenia. Mice treated with haloperidol recapitulate many of the features of TD, including jaw tremors, tongue protrusions, and vacuous chewing movements (VCMs), thus they are an ideal model system. In this study, we have employed the genetically diverse Collaborative Cross recombinant inbred inter-crosses (RIX) to elucidate the genetic basis of ADRs. We have performed a battery of behavioral tests (open field, extrapyramidal side effects, VCMs) in over 750 mice from 70+ RIX lines treated with haloperidol or placebo in order to monitor the development of ADRs. Strain effects were significant for every phenotype and interaction between strain and treatment was also highly significant across multiple behavioral measures. Understanding the genetic basis for the susceptibility to ADRs could lead to the development of safer and more effective therapeutic approaches for schizophrenia.

O-50: Many shades of gray: a comparative analysis of pheomelanin-specific mouse mutants

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The etiology of gray coat color is multifaceted with one phenotype class represented by a set of six classical mouse mutants including dwarf grey ($Ggt1^{dwg}$), grey-lethal ($Ostm1^{gl}$), subtle gray ($Slc7a11^{sut}$), *Clcn7*, grizzled (*gr*) and grey intense (*gri*). These mutations disrupt the production of a yellow pigment, pheomelanin, in the agouti band of the hair and are distinct from mutations like ashen ($Rab27a^{ash}$) that dilute eumelanin (black pigment) in the hair, or vitiligo ($Mitf^{mi\text{-}vit}$) that result in age-dependent loss of pigmentation. In humans, the ratio of eumelanin and pheomelanin produced by melanocytes directly impacts skin cancer risk; eumelanin provides protection from UV-induced DNA damage while pheomelanin has the opposite effect, potentially increasing the risk of UV-induced damage. Therefore, understanding the function and regulation of the genes that specifically disrupt pheomelanin production is of particular interest. Recently, we identified the deleterious mutation in grizzled mice ($Mfsd12^{gr}$), following a genome-wide association study linking the *MFSD12* locus to variation in skin pigmentation among African populations. To extend our knowledge of genes required for pheomelanin synthesis, we have used exome sequencing to identify a candidate mutation in *gri,* and are currently confirming the causative gene with a complementation test between a Crispr/Cas9-mediated *Usp32* knock-out allele and the original *gri* allele. Interestingly, mutations disrupting pheomelanin production are associated with pleiotropic phenotypes, including reduced viability, increased bone density, and delayed tooth eruption that vary among the mutants. Varied genetic backgrounds and a lack of comparative studies have made it difficult to distinguish cell-type specific gene function from the influence of genetic background on phenotypic variability. Thus, we have begun a comparative study with each of the mutants on a defined, uniform genetic background to assess the impact of each gene on pheomelanin production and to explore the reoccurring association between osteopetrosis and gray coat color.

O-51: Utility of the Pirc rat model of Familial Adenomatous Polyposis as a platform to test the role of the gut bacteria *Desulfovibrio vulgaris* on adenoma development

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Human epidemiological microbiome studies have identified several bacterial taxa associated with either the presence of colon cancer or a healthy gut. The challenge is to understand if these taxa are causative or merely associated with the presence of cancer, and to test them in appropriate model systems. In several studies examining tumor and healthy tissues from human colon cancer patients and controls *Desulfovibrio spp.* was associated with reduced adenoma burden. This bacteria has several unique attributes including sulfate reduction and the ability to form biofilms. To determine if this taxa has a protective role in tumor development, especially in a complex gut microbiota setting, we colonized Apc^{Pirc} rats with either a type-1 secretion system (T1SS) mutant that is incapable of forming a biofilm, or the wildtype *Desulfovibrio vulgaris* strain. We had determined that the T1SS is required for biofilm formation and may therefore affect colonization potential of the bacteria. We confirmed via qRT-PCR the presence of the wildtype strain in fecal and biopsy samples post-treatment, whereas the mutant strain was undetectable in most treated rats. At sacrifice, we found that the T1SS-competent strain suppressed tumor burden in the colon compared to rats treated with the T1SS-deficient strain or those treated with PBS (One-Way ANOVA, $p<0.05$). Sulfide and gene expression assays suggested that the increased tumor burden may be mediated through an increase in hydrogen sulfide production in the colon. 16S rDNA gene sequencing demonstrated significant bacterial population differences (PERMANOVA, $p<0.05$) between the groups at 2 months of age especially in the mucosal biopsies, suggesting a locally mediated protective effect of the GM community. These data emphasize the importance of bacterial colonization to modulate a host phenotype, and implicate the importance of spatial organization of bacterial populations in the gut to modulate the host genetic events leading to adenoma development.

O-52: Update on housing density studies using research mice: time for a change?

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Space recommendations for mice made in the *Guide for Care and Use of Laboratory Animals* have not changed since 1963, despite important improvements in husbandry and caging practices. The 1996 version of the *Guide* put forth a challenge to investigators to produce new data evaluating the effects of space allocation on wellbeing of mice. We have summarized many studies published in response to this challenge and find that they share similar outcomes. We distinguish between studies using ventilated or non-ventilated caging systems and those evaluating reproductive performance or general wellbeing of adult mice. We discuss how these studies might impact current housing density considerations in both production and research settings and consider gaps in mouse housing density research. Numerous reliable methods used to monitor and quantify general wellbeing of research mice, including behavioral and physiological assessments, are also included in our summary. Collectively, this large body of new data suggests that husbandry practices dictating optimal breeding schemes and space allocation per mouse can now be reconsidered. Specifically, these data demonstrate that 1) pre-wean culling of litters has no benefit; 2) trio breeding is an effective production strategy without adversely affecting pup survival and wellbeing; and 3) housing of adult mice at densities of up to twice current *Guide* recommendations does not compromise wellbeing for most strains.

We feel that many important studies carried out in response to a need for further assessment have since addressed floor space allocation, providing a substantial baseline of new information for evaluating appropriate policies. Moreover, because the many studies that have been carried out in the intervening years are consistent in their findings, this should eliminate any requirement to repeat such studies within each institution, thereby reducing the overuse of mice in research.

O-53: Misregulation of an activity-dependent splicing network impacts neuronal translation and underlies autism spectrum disorders

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Alternative splicing represents a key mechanism, which can increase the complexity of the transcriptome and thereby expand the repertoire of protein interactions and functions. We are analyzing how alternative splicing regulation underlies neurobiological development, function and disorders.

We have shown that, in the brains of a substantial proportion of autism spectrum disorder (ASD) individuals, a neuronal-specific alternative splicing network of microexons (short 3-27 nt exons) is misregulated and the expression of the neuronal microexon regulator SRRM4 (aka nSR100) is reduced (Irimia et al., Cell, 2014).To investigate whether nSR100 misregulation is causally linked to autism, we generated mutant mice with reduced levels of nSR100 and its target splicing program. Remarkably, these mice display hallmark ASD features, including altered social behaviors, synaptic density and signaling. Moreover, increased neuronal activity, which is often associated with ASD, results in a rapid decrease in nSR100 and splicing of microexons that significantly overlap those misregulated in autistic brains. Thus, misregulation of an nSR100-dependent splicing network controlled by changes in neuronal activity is causally linked to an important subset of autism cases (Quesnel-Vallieres et al., Mol. Cell, 2016).

To identify nSR100 target exons with significant neurobiological roles,we performed iCLIP-Seq experiments, which identified two conserved paralogue microexons in *Eif4g1* and *Eif4g3* translation initiation factors. Mutations that affect formation of an EIF4G-containing translational complex have been linked to ASD. Using CRISPR-based ablation and quantitative mass spectrometry, we observe that these microexons preferentially increase translation of proteins important for neuronal differentiation and synaptic function. Importantly, by generating mice deficient of each microexon, we observe that deletion of the *Eif4g1* microexon alone results in altered social behaviors, impaired learning and memory and disruptions in the excitatory-inhibitory synaptic balance and function.

These results place activity dependent microexon splicing upstream of key translational regulatory events and suggest possible therapeutic avenues to ameliorate ASD.

O-54: Machine learning predicts association between *Ythdf3* and cognition in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a complex trait likely modified by many genetic variants. In order to identify novel genes involved in modifying susceptibility to AD-related cognitive decline, we assessed cognitive function via contextual fear conditioning in a novel genetically diverse mouse model of AD. Using quantitative trait locus (QTL) mapping, we identified a QTL associated with contextual fear learning. As is typical with QTL mapping, this locus contained dozens of genes. Identifying the causal gene in this QTL experimentally could take years or decades and would be highly resource-intensive. Prioritization through literature searches is strongly biased by prior knowledge, and predictions cannot be systematically evaluated. Here we propose a computational method for prioritizing positional candidate genes. This method does not require auxiliary data, is minimally biased by prior knowledge, and systematically evaluates the strength of each prediction. The input for this method is a gene set that is related to the mapped trait. This gene set can be derived either experimentally or from public databases. We used a mediation analysis to generate a list of 1635 genes associated with learning from experimentally derived RNA-seq data. We trained support vector machines (SVMs) to distinguish these trait-associated genes from genes randomly selected from outside the set. We then used the trained models to quantify the extent to which each positional candidate gene was associated with the gene set. *Ythdf3* was the top-ranked gene. Virtually un-annotated, this gene is nevertheless a plausible candidate. *Ythdf3* is known to be involved in translation of m6A-modified mRNAs in neurons and may be associated with neuro-regeneration. We hypothesize that *Ythdf3* plays a role in the decline in learning seen in AD. Experimental validation of this hypothesis will demonstrate the ability of this computational prediction method to enhance the discovery of molecular mechanisms underlying complex traits.

Poster Abstracts

P-1: INFRAFRONTIER – resources and services to advance the understanding of human health and disease using mammalian models

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INFRAFRONTIER is the European Research Infrastructure for the development, phenotyping, archiving and distribution of model mammalian genomes. The INFRAFRONTIER Research Infrastructure provides access to first-class tools and data for biomedical research, and thereby contributes to improving the understanding of gene function in human health and disease using mice. The INFRAFRONTIER network currently consisting of 29 partners is engaged in several EC funded projects, such as INFRAFRONTIER2020, IPAD-MD and CORBEL, andcontributes to the International Mouse Phenotyping Consortium (IMPC).

The core services of INFRAFRONTIER comprise model generation, specialised phenotyping services, systemic phenotyping of mouse mutants in the participating mouse clinics, as well as archiving and distribution of mouse mutant lines by the European Mouse Mutant Archive (EMMA). In addition, INFRAFRONTIER offers specialized services, such as the generation of germ-free mice (axenic service) and training in state-of-the-art cryopreservation and phenotyping technologies. Reduction and refinement to improve animal welfare are among the major goals of INFRAFRONTIER's technology development programme.

The EMMA branch of INFRAFRONTIER offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. EMMA currently holds nearly 6000 mutant mouse strains, half of which have been produced from the International Mouse Knockout Consortium (IKMC) resource. The EMMA network is comprised of 16 partners from 13 countries who operate as the primary mouse repository in Europe. EMMA is funded by the partner institutions and national research programmes. Information on mouse strain submission and ordering and all other services offered by INFRAFRONTIER can be accessed online at www.infrafrontier.eu.

TS-5, P-2: Investigating genetic diversity of centromeres: a repetitive, non-coding, unassembled region of the genome

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An enduring biological puzzle is why many genetic loci with conserved biological functions are rapidly evolving at the sequence level. We are investigating this apparent paradox using mammalian centromeres as a case-study.

Centromeres are composed of large tandem alpha satellite repeat arrays that serve as binding motifs for the centromeric histone proteins that epigenetically specify these critical chromosomal domains. Centromeres provide a platform for the assembly of the kinetochore machinery that drives chromosome segregation during cell division. Despite their well-conserved functional roles, there is remarkable centromere sequence variability between species, between individuals, and even among chromosomes in a genome. Due to the satellite repeat composition of these loci, they remain gapped on every high-quality mammalian genome assembly. The absence of centromere reference sequence hampers efforts to elucidate the full scope of diversity across these regions, infer mechanisms of their evolution, and link genetic variation across these regions to specific functional consequences.

We are cataloging diversity across centromeric sequences in genetically diverse house mice (*Mus musculus*) using a two-pronged approach. First, we are harnessing the site-specificity of CRISPR gene editing to excise single centromere containing fragments from the genome, followed by isolation for targeted long read sequencing using pulse-field gel electrophoresis. Second, we are combining publicly available mouse genome data with a k-mer based computational strategy to bioinformatically define the repeat composition of centromeres. By determining the sequence of centromeres from diverse mouse strains and subspecies, our work stands poised to shed light on the mutational mechanisms by which diversity across these regions emerges, uncover the evolutionary processes that drive their rapid evolution, reveal their roles in chromosomal rearrangements genome evolution, and facilitate functional tests of their effects on cancer, infertility and meiotic drive.

P-4: Applying genome editing technology to mutant mouse generation and mouse resource archiving in RIKEN BRC

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RIKEN BioResource Research Center (BRC) participates in the International Mouse Phenotyping Consortium (IMPC) which is currently composed of 18 research institutions [1]. Early efforts at several IMPC centers targeted critical exons to introduce small insertions or deletions (indels) by imprecise non-homologous end-joining repair of Cas9-mediated double strand breaks. Because of their random nature, indel alleles are difficult to screen and quality control and therefore cannot be standardized. The IMPC shifted its efforts to produce alleles that more closely resembled knockout alleles made in ES cells by using Cas9 to generate exon deletion alleles. The approach that combines paired gRNAs and Cas9 would be reliable, cost-effective and efficient for producing knockout mice for phenotyping.
We will show our recent progress on 1) expanding the approach to electroporation of CRISPR components into fresh or thawed *in vitro* fertilized C57BL/6N (B6N) zygotes [2], which can be a convenient and efficient alternative approach to microinjection for the IMPC mouse production, 2) expanding the allele types of several genes (cre knock-in or conditional knockout mice in a B6N background) to which Japanese scientific community is paying increasing attention, and 3) expanding the list of genetic background strains in which mouse genome editing is feasible by using MSM/Ms, a wild-derived inbred strain originated from Japanese wild mice *Mus musculus molossinus* [3-5]. MSM mouse strain has several unique phenotypes such as small body size, low incidence of tumor, and distinct behaviors, positioning itself as a promising resource not only for uncovering novel functions of the mouse genome but also for offering insights into pathophysiology of human diseases.

Reference

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P-5: Origin and Fate of *de novo* Insertions of Transposable Elements in the Collaborative Cross

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Transposable elements (TEs) represent a large fraction of the mammalian genome and *de novo* insertions are known to contribute to disease causing mutations. By combining standard whole genome sequencing and a novel computational pipeline to the Collaborative Cross (CC) we have recently identified dozens of *de novo* TE insertions in this genetic reference population. The genomic context of these insertions is significantly different that the one observed for older TEs, strongly suggesting that many may have deleterious effects in gene function. If this hypothesis is correct, highly deleterious mutations should be purged from the corresponding CC strain while neutral mutations may rise in frequency and become fixed. Our extensive collection of CC samples from early generations to mice alive in the current colony provides an outstanding experimental setup to determine the origin and evolutionary trajectory of each mutation and determine whether there is evidence for selection. We selected four available CC strains with at least 9 *do novo* events (CC003/Unc, CC005/TauUnc, CC027/GeniUnc and CC055/TauUnc) and two CC strains that are not available from the UNC core (CC020/GeniUnc and CC074/Unc). We determined the origin and trajectory by genotyping a large cohort of mice of each strain with birthdates spanning from 2010 to 2018, including the most recent common ancestors of each strain. Our preliminary results indicate that *de novo* TEs originate at different generations within each pedigree and that, as predicted, there is a wide range of allele frequencies (5-100%). We will present detailed results for over 50 *de novo* TEs including some with strong deleterious effects.

P-7: RNA-Seq Technologies Identify Causal Mutations in Mice with Mendelian Disorders

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Exome and whole genome sequencing have been the mainstays for identifying causal mutations in rare Mendelian disorders of humans and mice. These technologies, while highly valuable, still only allow us to identify causal mutations with a success rate around 50%. In The Jackson Laboratory's Comparative Mendelian Disease Genomics (CMDG) program (R24 OD021325/OD/NIH HHS/United States), we are exploring alternative technologies to identify disease-causative mutations where whole-exome sequencing alone has been insufficient. One such technology is transcriptome sequencing (RNA-Seq). Here, we report the analysis of sequenced transcriptomes from disease-relevant tissues for over forty mouse models across a broad spectrum of Mendelian disorders (*e.g.*, those affecting craniofacial development, metabolism, neurological function, pigmentation, skeletal morphogenesis, *etc.*). Our preliminary analysis of 15 strains highlights many instances where transcriptome sequencing has succeeded in identifying disease-causative mutations where other technologies have not, and suggests a further role for RNA-Seq, not only for mutation discovery in model organisms, but also in the diagnosis of Mendelian disease among patient populations. Our latest analysis, focused mainly on identifying splicing mutations, will describe the complete findings for all 40+ mouse strains.

P-8: Alliance for Genome Resources: Visions for the Future from a Mouse Perspective

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The Alliance for Genome Resources (www.alliancegenome.org) provides a comparative and functional genetics and genomics portal for data associated with the major model organism resources (MGD, SGD, FlyBase, WormBase, ZFIN, RGD, and the GOC). The Mouse Genome Database (MGD) (www.informatics.jax.org) group actively participates in these Alliance developments. These resources work together to provide shared access to these data through the Alliance web site or via APIs. The goal is to provide integrated access to these data especially in support of the model organism contributions to the study human biology and disease. The next major release of the Alliance web site will be in October, 2018, and will include enhanced integration of disease and phenotype data, expression data, comparative variant data, and functional summaries for genes.

The Mouse Genome Database (MGD) group actively participates in Alliance development. An important responsibility of the Alliance community is to serve as a major data steward to the NIH Data Commons Pilot Project, providing the suite of model organism data for analysis in the NIH cloud. As part of the Commons Cloud Environment, the Alliance is working to implement GO enrichment and Disease Navigator tools, with MGD being co-developer with Rat Genome Database on the Disease Navigator tool. Regular updates to MGD within the Mouse Genome Informatics environment continue to support the scientific research community from both MGI and Alliance perspectives.

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P-9: Content and performance of MiniMUGA, a new tool to improve rigor and reproducibility in mouse research

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The laboratory mouse is the most popular animal model for biomedical research, due in part to its well annotated genome, wealth of genetic resources and the ability to manipulate its genome. Despite the importance of genetics for mouse research, genetic QC is poor in part due to the lack a cost effective and robust platform. Genotyping arrays are standard tools for mouse research and remain an attractive alternative in the era of high-throughput whole genome sequencing. Here we describe the content and performance of MiniMUGA, an array based genetic QC platform with approximately 11,000 probes. In addition to robust discrimination between most laboratory strains (classical and wild-derived), MiniMUGA was designed to contain several features that are not available in other platforms: 1) probabilistic chromosomal sex determination, 2) discrimination between substrains and strains from different commercial vendors, 3) diagnostic SNPs for a large set of popular laboratory strains and 4) detection of constructs used for genetically engineered mice. An easy to interpret genetic QC report summarizes the results. To determine the performance of MiniMUGA we genotyped over 5,000 samples from a wide variety of backgrounds. The performance of MiniMUGA compares favorably with three previous iterations of MUGA arrays both in discrimination and robustness, with a much richer annotation that should simplify custom analyses by individual researchers. Among the additional findings in this initial study is the detection of a substantial number of XO and XXY individuals, the ability to the extend the successes of reduced complexity crosses to genetic backgrounds other than C57BL/6, and the robust detection of dozens of constructs. We have also generated publicly available consensus genotypes for over 200 inbred strains. We conclude that MiniMUGA is an outstanding platform for genetic QC and important new tool to increase rigor and reproducibility of mouse research.

P-10: Testing pleiotropy vs. separate QTL in multiparental populations

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Multiparental populations, such as the Diversity Outbred mouse population, are a new resource for systems genetics studies. Distinguishing close linkage of distinct quantitative trait loci from one pleiotropic locus that associates with multiple traits has implications in biomedical research, plant and animal breeding, population genetics, and other genetics applications. Presence of two distinct loci potentially enables selective modification of one locus at a time. In the case of a single pleiotropic locus, it may be difficult or impossible to modify the locus without influencing both traits. We extend methods of Jiang and Zeng (1995) to develop a likelihood ratio test for the alternative hypothesis of close linkage of two loci against the null hypothesis of pleiotropy for a pair of traits that map to a single genomic region. Unlike previous tests of these competing hypotheses, our test incorporates polygenic random effects to account for complex patterns of relatedness among subjects. Additionally, our test accommodates more than two founder alleles. We use a parametric bootstrap to determine statistical significance of likelihood ratio test statistics. We characterize our test's type I error rate and power to detect close linkage of two loci in simulation studies, where we find that it is slightly conservative and has reasonable power when the univariate LOD peaks are strong. To demonstrate its practical utility, we apply our test to data from a study of 261 Diversity Outbred mice. We perform pairwise analyses of three traits that map to a single region on Chromosome 8. We find evidence for two distinct QTL in the region. We share our methods in a freely available software package (https://github.com/fboehm/qtl2pleio) for the R statistical computing environment.

TS-1, P-11: Whole Genome Sequence Analysis Issues Revealed by Evaluation of a Linkage Peak in a Dominant ENU Suppressor Mouse Line

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We previously established a large multigenerational mouse line harboring a dominant, highly penetrant (72.4%) ENU-induced mutation that suppresses the $F5t^{m2Dgi}/F5^{tm2Dgi}$ $Tfpi^{tm1Gjb}/+$ perinatal lethal blood clotting (thrombosis) phenotype. We mapped this putative suppressor mutation to a 7.5Mb region on Chromosome 3. This region contains the essential thrombosis gene *F3*. We previously demonstrated that *F3* haploinsufficiency potently suppresses $F5t^{m2Dgi}/F5^{tm2Dgi}$ $Tfpi^{tm1Gjb}/+$ lethality, so an ENU-induced mutation affecting *F3* expression/function would be a prime thrombosuppressor candidate. However, whole exome sequencing (WES) revealed no coding mutations in *F3* or any other gene in the candidate interval. In addition, exomic mutations elsewhere in the genome did not exhibit a significant segregation with the lethal phenotype. This suggests an uncharacterized, non-exomic *F3* regulatory mutation as the thrombosuppressor. To identify candidate variants, we performed whole genome sequencing (WGS) on five $F5t^{m2Dgi}/F5^{tm2Dgi}$ $Tfpi^{tm1Gjb}/+$ mice from our line. WGS data were aligned to the C57BL/6J reference genome and variants called using an established bioinformatics pipeline. We identified 31 candidate variants within the linkage peak region: 24 SNPs and 9 small indels. None of the variants were exomic, consistent with our previous WES analysis. Of the 31 variants in the 7.5Mb region, Sanger re-sequencing analysis revealed 8 of these variants as miss-calls and the remaining 23 were unable to be re-sequenced due to the highly repetitive nature of the DNA sequences. This high number of false-positive calls possibly results from misalignment of the mixed strain background to the reference genome. The inability of our WGS analysis to identify the thrombosuppressor could potentially be due to false-negative calls resulting from misalignment, and this situation could be exacerbated by short read sequencing. We are presently exploring different WGS analysis options, including *de novo* genome assembly, to minimize misalignment issues and enable us to identify the thrombosuppressor mutant.

P-12: Genetic variation in opiate induced respiratory depression in mice

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Prescription of opioids, a class of drugs used to reduce pain, has nearly quadrupled from 1999 to 2014 leading to an epidemic in addiction and overdose deaths in the United States. Opioid overdose is the result of respiratory depression followed by hypoxia, hypercapnia and is followed by death. There is substantial inter-individual variability in response to opioids. Understanding the mechanisms and predictors of overdose and vulnerability to overdose will be critical to reducing the occurrence and impact of these events. Research in laboratory animals can characterize mechanisms of overdose liability in detail. Genetic analysis in the laboratory mouse, with exquisite precision, diversity and environmental control can identify variable mechanisms of respiratory depression and lethality associated with opioid overdose. Although there is some evidence for strain differences in sensitivity to overdose, the effects of genetic variation on morphine overdose susceptibility has been poorly characterized to date. Determining precise quantitative metrics associated with lethality is necessary to facilitate genetic dissection of the mechanisms underlying susceptibility and resistance. We have adapted piezoelectric sleep monitors to detect respiratory rhythms, and have defined an automated high-throughput technology to monitor the respiratory depression associated with morphine administration. Using the eight progenitor inbred strains of the Collaborative Cross, we have detected heritable variation in morphine $LD_{50.}$ The LD_{50} among strains ranged from 225 mg/kg - 882 mg/kg with several strain × sex interactions. Using the piezo data we were able to determine the survival time and recovery time for each strain. These quantitative measurement were used to calculate the heritability of these morphine sensitivity traits (time to death ICC= 0.338, time to recovery ICC= 0.345). We have begun a biased genetic approach to map the genomic regions responsible for the phenotypic variation in respiratory physiology and response to morphine in the Diversity Outbred population

TS-6, P-13: Cellular systems genetics implicates KRAB zinc finger proteins in mediating variation in chromatin state in pluripotent stem cells

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Chromatin state modulates genome function permitting genetic information to be precisely interpreted at specific times throughout development. Determining how chromatin state is established is critical in understanding the mechanisms controlling specification and maintenance of cell identity. How genetic variation impacts chromatin regulation during development, leading to downstream changes in gene expression and ultimately phenotypic variation, is poorly understood. To address these questions we have taken a cellular systems genetics approach to early development using mouse embryonic stem cells (mESCs) as a model system. We derived a mapping panel of mESCs from 33 BXD recombinant inbred lines originating from a cross between C57BL/6J (B6) and DBA/2J (D2) mouse strains. Measuring chromatin accessibility (ca) and gene expression (e) profiles in this mapping population grown in conditions maintaining the ground state of pluripotentcy, we identified large-scale cis and trans quantitative trait loci (QTL). Many eQTL and caQTL map to six major distal loci, indicating a common regulatory system driving changes in chromatin and gene expression. Interestingly, most distal QTL correspond to locations of genes encoding KRAB zinc finger proteins (KRAB-ZFPs). We observe differential expression of transposable elements (TEs) between B6 and D2 mESCs, which are known targets of KRAB-ZFPs. Importantly, in the BXD mESCs, the genetic control of many TEs map back to the same caQTL. Current effort is focused on validating KRAB-ZFPs and associating functional elements regulated by caQTL with TE integration and nearby eQTL. Together these data support a model of evolutionary conflict between TE integration and divergence in the KRAB-ZFPs that together drive the re-wiring of gene expression networks in ESCs.

TS-3, P-14: A mouse translocation dysregulating topologically – associating *Galnt17* and *Auts2* genes associated with behavioral and neuropathological phenotypes related to the human AUTS2 syndrome

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Many studies have shown human diseases can be caused by mutations in non-coding genomic regions, including promoters, intergenic enhancers, and other elements that regulate transcription of nearby genes. These elements and the genes they regulate are organized into topologically associating domains (TADs), with elements within the TADs potentially able to regulate multiple genes. Here, we describe a mouse model, 16Gso (T(5:8)16GSO), carrying a chromosomal translocation between Chromosomes 5 and 8 and exhibiting a complex suite of morphological and behavioral phenotypes. We mapped the translocation breakpoint between two genes, *Auts2* and *Galnt17*, located together within a conserved TAD, and show that the two genes expressed together in the same neuron and glial populations in developing brain. *AUTS2* region mutations have been linked to a wide variety of neurodevelopmental and neuropsychiatric diseases, and most studies have focused on *Auts2* functions. However, most mutations are genomic rearrangements, raising the potential for involvement of *Galnt17*. We found that 16Gso mice express many symptoms related to those in human AUTS2 syndrome patients including craniofacial abnormalities, developmental delay, learning deficits, repetitive behaviors, and an abnormal response to novelty; the mutants also display specific neurodevelopmental pathologies, and neurons in culture show related cellular defects. RNAseq experiments confirmed that both *Auts2* and *Galnt17* are dysregulated in 16Gso mutants, indicating that both genes may contribute to the mutant phenotypes. We are examining the role of each gene in these phenotypes using genetic complementation, and working to decipher relationships between noncoding elements and genes within the *Auts2*-*Galnt17* TAD. These studies will provide novel insights to the genetic and molecular basis of the wide range of phenotypes associated with *AUTS2*-region mutations, and a new model for TAD-linked multigenic disorders.

TS-11, P-15: Alterations in metabolism and circadian rhythm in a *Pax6* mutant mouse line

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The transcription factor paired box protein 6 (PAX6) is a pleiotropic factor that is involved in the development of brain, central nervous system, eye and the pancreas. Its role in maintenance of pancreatic islet homeostasis and metabolism has been a subject of a few recent studies. Interestingly, PAX6 is not only involved in the development of the pineal gland, the main regulator of diurnal rhythm, but several patients carrying *PAX6* mutations show hypoplasia or absence of the pineal gland. Therefore, such patients may present several phenotypes including pineal gland agenesis. However, much less is studied in this regard, possibly due to such features being masked by more conspicuous diseases such as overt diabetes and eye defects.

Here, we investigated an ENU-generated *Pax6* mutant mouse line, which displays eye defects and metabolic alterations. Most strikingly, homozygous mutant mice failed to display metabolic flexibility and showed a lack of typical diurnal changes in metabolic rate, strongly indicating altered circadian rhythm. This was further accompanied by changes in feeding behavior, where mutant mice consume more food during the inactive phase as compared to their wild type littermates. Moreover, we found changes in the expression of circadian genes such as *Clock*, *Nr1d1* and *Per1* in the liver, suggesting altered physiology may arise from disturbances in activation and repression cycles. Furthermore, presence of the pineal gland was observed in homozygous mutant mice however, defects in the eye suggest a compromised visual pathway that may also affect non-visual responses such as sleep regulation and entrainment of circadian rhythm. Hence, this study aims to illuminate an interaction between circadian rhythm and metabolism with regards to the function of PAX6.

P-16: The role of genetic variation in defining the regulatory landscape of the *in vivo* ER stress response

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The phenotypic expression of disease varies among individuals. Differences in genetic background among patients is a major contributor to these differences in disease outcomes. We are studying how natural genetic variation among individuals contributes to differences in disease outcomes by incorporating inter-individual genetic variation in the context of endoplasmic reticulum (ER) stress. The ER is an organelle involved in protein folding. ER stress is the result accumulation of misfolded proteins in the lumen of the ER. If unresolved cell death and disease occurs. ER stress is an important component to many diseases and can impact disease outcomes. We hypothesize that inter-individual genetic variation in the ER stress response pathway is an important contributor to variable disease outcomes. We have previously shown that the ER stress response is highly variably across different genetic backgrounds. Our goal is to uncover how genes and genetic variants of the ER stress response contribute to a variable response. We are performing an *in vivo* study that will determine how the *cis*- and *trans*- regulatory landscape drives variation in the ER stress response in a tissue-specific manner. We are using C57BL/6J and CAST/EiJ, two genetically divergent strains. These strains are crossed to produce F1 mice. For each response gene, we can identify *cis*- and *trans*- regulatory effects by comparing the relative allelic expression in the F1 to the ratio of expression between the parental strains. We are inducing ER stress, performing RNA-seq on multiple tissues, and analyzing the ER stress regulatory landscape and allele specific expression in a strain and tissue dependent manner. We are using the *Drosophila* to test putative genetic modifiers of the ER stress response. An ER stress response modifier gene could be a therapeutic target in a number of diseases with a strong ER stress component.

P-17: Genetic modification in the rat model: Streamlining new genome editing techniques

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The rat is re-emerging as an increasingly popular animal model species in biomedical research. Manipulation of the rat genome has recently become achievable at high efficiency due to the use of engineered nucleases, such as CRISPR/Cas systems. Although using many of these new genome editing systems is now rudimentary, there are still technical aspects involved in generating genetically modified rats that limit throughput. For example, delivery of CRISPR/Cas reagents (or other genome editing reagents) to rat zygotes often involves challenging microinjection procedures that require expensive micromanipulation equipment. Furthermore, transferring the manipulated embryos into pseudopregnant surrogate dams requires anesthetizing rats in conjunction with a specialized surgical procedure. Here, we have: 1) optimized embryo electroporation parameters to deliver CRISPR/Cas9 reagents for high-throughput generation of knockout and knockin rat models, 2) refined rat embryo culturing techniques to allow for efficient *in vitro* blastocyst development, and 3) adapted a non-surgical embryo transfer (NSET) method to transfer manipulated embryos into pseudopregnant surrogate rats. Together, these procedures streamline the ability to efficiently generate rat models in a straightforward and high-throughput manner.

TS-12, P-18: Pairing systems genetics and longitudinal analyses to identify regulators of complex lung disease phenotypes

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Mouse models of lung disease have been instrumental to our understanding of pathogenesis and have important roles in addressing existing unmet therapeutic needs. Given that many lung diseases are heterogeneous and driven by numerous genetic and environmental factors, the use of multi-parental mouse populations to facilitate robust identification of mechanisms regulating such complexity is warranted. We employed a systems genetics approach with the Collaborative Cross mouse population to overcome existing challenges in elucidating the molecular mechanisms driving airway remodeling in the context of asthma. Airway remodeling refers to alterations to the cellular and tissue composition of the airways that often cannot be fully resolved and may contribute to asthma attack fatality. We chronically exposed 31 Collaborative Cross (CC) strains and BALB/cJ mice to house dust mite allergen to induce features of airway remodeling that mirror human disease. CC strains exhibited a range of responses to chronic allergen exposure as determined by airway inflammatory profiles, airway mucus content, and quantitative and semi-quantitative morphological analysis of airway tissue for goblet cell metaplasia, subepithelial fibrosis, and smooth muscle hyperplasia. Of note, the correlations among these phenotypes indicated both distinct and shared genetic regulation of disease traits. RNAseq of isolated airway tissue will provide insight on the transcriptional patterns underlying disease severity, aid in prioritizing candidate phenotypic regulators, and be used to determine how mouse model transcriptional profiles align with existing human disease samples. A subset of CC strains will be selected for a time course of allergen exposure to investigate the signaling events controlling remodeling initiation and progression. This dataset will be the foundation for robust airway remodeling analyses that are currently intractable in the human population and are critical steps in identifying potential therapeutic targets.

P-19: Zebrafish Crispr/Cas9 screen for cross-species functional conservation of genes regulating mouse pheomelanin synthesis

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In mammals, pigmentation is produced in a specialized cell called a melanocyte, which produces both eumelanin (black pigment) and pheomelanin (red/yellow pigment). Eumelanin is protective against UV radiation, while pheomelanin is associated with oxidative stress; individuals with higher pheomelanin levels have a propensity for sun-induced skin damage and a higher skin cancer risk. A previous human GWAS study identified a novel gene, *MFSD12*, that is associated with skin pigmentation in African populations. CRISPR/Cas9-mediated knockout of the mouse *Mfsd12* gene on an agouti background resulted in a uniformly gray coat color due to the loss of pheomelanin in the hair shaft. Interestingly, knockout of the *Danio rerio* (zebrafish) homolog, *mfsd12a*, also disrupted pigment cell function. Unlike mammals, zebrafish have three different pigmentation cell types that produce different pigments: melanophores (black), xanthophores (yellow), and iridophores (silver). Interestingly, the *mfsd12a* knockout specifically affected xanthophores. Even though the yellow pigment produced in xanthophores is chemically different than pheomelanin, the *mfsd12a* phenotype suggests that the genetic pathways regulating yellow pigment production may be conserved across species. Thus, we are currently screening a broad set of 11 candidate genes in a comparative study to evaluate their functional conservation across species. Preliminary results in zebrafish have identified a novel xanthophore phenotype in *colony stimulating factor 1a* (*csf1a*) knockouts and also suggested that *Mfsd12* may be unique in cross-species regulation of yellow pigment production. Further studies are in progress to characterize the xanthophore phenotype of the *csf1a* zebrafish mutant. Examining the genes necessary for yellow pigment production across species will increase our understanding of the genetic regulation of human pigment variation and associated skin cancer risk, as well as provide animal models for future studies of drug intervention.

P-20: Fast identification and validation of causal variants using whole genome sequences of commercially available mouse substrains

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The mouse is the premier mammalian model organism of human disease, in part, due to the wealth of inbred strains commercially available, as well as extensive genomic information. Despite the widespread usage of closely related inbred strains (i.e. substrains) from different vendors, relatively little is known about genetic differences segregating between these strains. Such differences can lead to phenotypic differences between substrains, which have contributed to issues impacting reproducibility in scientific studies. Here we report on the genetic variation segregating within 8 commercially available substrain groupings (129, A/J, BALB/c, C57BL/6, C3H/He, DBA1, DBA2, and FVB), including 37 novel whole-genome sequences of inbred mouse strains. We identify genetic variants (SNPs and small indels, larger deletions and actively segregating transposable elements) segregating between substrains across the genome. These variants allow for the extension of reduced complexity crosses, genetic mapping populations between substrains, to multiple pairs of substrains. They also provide a catalogue of genetic variants across independent strains which can be used for validation of causal variants. Lastly, these data help to clarify the phylogenetic relationships between mouse substrains.

P-21: The German Mouse Clinic: a collaboration-based platform for phenotype characterization

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The German Mouse Clinic (GMC, www.mouseclinic.de) is a well-established collaboration-based platform for phenotype characterization of mouse lines. In addition to a comprehensive phenotypic analysis of mice for the scientific community, more and more special and sophisticated scientific questions need to be resolved. Therefore, the GMC recently established specialized phenotyping pipelines to address individual projects by a tailor-made phenotyping strategy. There are fourteen pipelines with special focus on the analysis of the neuronal and brain function, metabolism, respiratory system and immunity. For a general, broad analysis, there is a screening pipeline that covers the key parameters for the most relevant disease areas. This pipeline is similar to the International Mouse Phenotyping Consortium (IMPC) phenotyping pipeline, but covers additional disease areas (e.g. skin health) and contains additional metabolic phenotyping tests. An additional variant of this pipeline is applied for the analysis of aged mutant mouse lines, where the GMC will contribute 25 lines to the IMPC late-onset phenotyping program. All pipelines have been approved by the ethics committee of the local Government. We report our experiences concerning the users demand by the scientific community for the different special phenotyping areas, and we will show as an example for the application of our phenotyping pipelines “Memory impairment” and “Motor disorders” published data from phenotyping an ALS mutant mouse line.

O-12, P-22: Identification of meiotic non-crossovers in mice

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Programmed DSBs induced by SPO11 are required for successful pairing of homologous chromosomes in meiotic prophase I. Their position is mostly determined by the binding affinity of the zinc-finger methyl-transferase protein PRDM9, trimethylating H3K4 and H3K36. All DSBs need to be repaired, either by crossover (CO, with an exchange of chromatid arms), or by non-crossover (NCO, with no exchange), both being accompanied by gene conversions. Even though NCOs are more frequent (90%) than COs (10%), their genome-wide/chromosome-wide detection has not been shown yet in mice.

We detected ~100 of NCO gene conversions on ten mouse autosomes using WGS of corresponding C57BL/6J-Chr #PWD chromosomal substitution strains and their progenitors C57BL/6J (B6) and PWD/PhJ (PWD). This model combines the benefits of high diversity of the *Mus musculus domesticus* and *Mus musculus musculus* genomes and the 10-fold accumulation of gene conversion events during construction of a chromosome substitution strain. Based on the converted and surrounding non-converted SNPs we were able to identify gene conversions shorter than 100 bp as well longer than 200 bp.

We found PRDM9^{B6} and PRDM9^{PWD} binding motifs within 250 bp from the NCO gene conversions. Additionally, NCOs overlapped the known H3K4me3 modifications and DMC1 protein peaks from the ChIP-seq data of B6, PWD, (B6xPWD)F1, (PWDxB6)F1 genotypes, proving the meiotic origin of the observed gene conversions. A comparison of the origin of NCOs supported the idea of the asymmetry of PRDM9 binding. The gene conversions led to erosion of the PRDM9 binding sites and to a GC bias ($P < 0.001$). Interestingly, the GC bias was also present in the surrounding 800 bp, suggesting the presence of historical recombination.

P-23: Genome sequencing of PL/J, QSi3, and QSi5 identifies novel coding and truncating variants for divergent litter rearing capacity

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Enormous effort has gone into the identification of quantitative trait loci for milk production and composition in dairy animals. These traits have significant economic importance and implications for human health. Prior work by our group has used the mouse diversity panel to identify phenotypic extremes and map QLT for lactation-related traits. This work was limited by a lack of genetic data in key strains with high and low litter-rearing capacity. The genomes of females from two low lactation strains, the PL/J, and QSi3, and a high lactation strain, the QSi5, were sequenced to 36-, 43-, and 40-fold coverage, respectively. This data generated a catalog of single nucleotide polymorphisms (SNP) and small insertions and deletions (INDEL) and identify private alleles for each of the strains. There were 33,581, 25,236, and 28,553 private SNP in PL/J QSi3, and QSi5, respectively. Of these, there were 221, 200, and 85 that overlapped with and produced non-synonymous or truncating mutations in the coding regions of 113, 100, and 49 genes, respectively. The most notable mutations were within the *Wnt2b* and *Acss3* genes in PL/J. Pathway analysis identified enrichment for immune-related pathways in QSi3. Enrichment for transport, lipid metabolism, and Wnt signaling was found in QSi5. As a second approach the list of genes affected by all mis-sense mutations present in the low lactation strains but not in QSi5, was intersected with differentially expressed genes identified through a comparison of RNA-Seq data from lactating mammary tissue of PL/J and QSi5. In this subset there were 438 differentially expressed genes. Pathway enrichment analysis of this list identified enrichment for L-cysteine degradation, folate transformations I, NR4A1 (Nur77) signaling in lymphocytes, Th1 pathway, and calcium-induced T lymphocyte apoptosis. Further comparisons combining lists of novel alleles in additional high and low lactation strains will inform future functional experiments.

P-24: Methyl-CpG binding domain protein 1 polymorphisms impact immune homeostatic levels of IgG1

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Host immunity plays a key role in protection against infection, however, there is significant individual-to-individual variation in the induction and quality of immune response that is driven in part by genetic factors. A growing body of evidence suggests that variation in immune status prior to specific insult (immune-homeostasis) may influence subsequent response to vaccination or pathogen challenge. Therefore, understanding genetic control of immune-homeostasis is necessary for assessing variability in immune response to vaccination or pathogen challenge. The Collaborative Cross (CC) is a genetically diverse mouse genetic reference population that was designed to mimic aspects of the genetic and phenotypic diversity that is observed in humans and can be used to mechanistically investigate the contribution of genetics to complex phenotypes. We investigated and genetically mapped variation in immune-homeostatic antibody levels in 59 CC strains. We measured antibody levels for IgM, IgG, and each of the following IgG subtypes: IgG1, IgG2a, IgG2b, IgG2c, and IgG3 by ELISA and mapped quantitative trait loci (QTL) associated with variation in antibody concentrations. We observed substantial variation in all antibody isotypes measured. We also identified a genome-wide suggestive ($p<0.1$) QTL on Chromosome 18 associated with baseline variation in IgG1. Haplotype effects show that WSB/EiJ, CAST/EiJ, and C57BL/6J alleles at the locus of interest are linked to higher levels of IgG1. Subsequent analysis of genes under the QTL identified Methyl-CpG binding domain protein-1 (*Mbd1*) as the most promising candidate associated with IgG1 variation. MBD1 regulates transcription through binding of methylated CpG dinucleotides and recruiting histone methyltransferase, SETDB1, to repress transcription through the induction of heterochromatin. Ongoing studies are evaluating the role of variation in *Mbd1* in the regulation of IgG1 production.

P-25: Transcriptional Profiling of a Pre-Symptomatic Rett Syndrome Mouse Model

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Rett syndrome (RTT) is a rare neurological disorder, with the vast majority of cases caused by sporadic mutations in the X-linked gene methyl-CpG-binding protein 2 (*MECP2*), a ubiquitously expressed master transcriptional modulator. Girls with RTT develop normally for approximately 6-18 months, followed by developmental stagnation and regression. Symptoms often include stereotypic hand movements, seizures, respiratory abnormalities, motor impairment, and autism spectrum-like behavior. Several mouse models recapitulate the RTT phenotype. Despite heterozygous (*Mecp2*/+) female mice being more clinically relevant, hemizygous males (*Mecp2*/Y) are often the preferred model as they exhibit a more penetrant phenotype. Although RTT neuropathology and transcriptional changes have been examined in symptomatic *Mecp2*/Y mice at time points of 6 - 8 weeks of age, these changes may be downstream effects of the diseased state. In spite of years of study, the precise molecular events that initiate RTT are largely unknown and treatment options for patients remain limited. To distinguish the transcriptional changes that occur exclusively due to the loss of *Mecp2* as a transcriptional regulator rather than being altered as a result of the diseased state, RNA-sequencing was performed on five specific brain regions of pre-symptomatic and symptomatic 129S6SvEv/Tac-*Mecp2*$^{tm1.1Bird}$/Y mice with their age-matched wildtype littermates. Already, numerous misregulated molecular pathways have been identified in distinct brain regions solely at the pre-symptomatic time point. Following validation, this outcome will guide single cell RNA-sequencing to identify the specific cell type and primary perturbations that initiate the onset of RTT. The findings will provide insight into both the early pathophysiology of RTT and normal *MECP2* function. It may also identify subsets of genes that are amenable to manipulation, and thus could reveal novel therapeutic targets, such as repurposing existing pathway-modulating compounds for early RTT treatment.

P-26: Expression of lncRNAs in ovarian cancer-associated fibroblasts is prognostic for patient survival

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Background: The tumour microenvironment is essential for the growth and metastasis of many solid tumours, including ovarian cancer. Cancer-associated fibroblasts (CAFs) represent the most abundant cell type in the tumour microenvironment and are responsible for producing the desmoplastic reaction that is a poor prognostic factor in ovarian cancer. Long non-coding RNAs (lncRNAs) have been shown to play important roles in several diseases, including cancer. However, very little is known about the role of lncRNAs in the tumour microenvironment.

Aim: To identify CAF-derived lncRNAs whose expression are associated with patient survival and use computational approaches to predict their function.

Methods: CAFs were microdissected from 67 ovarian tumours and RNA extracted. Gene expression was analysed using Affymetrix U133 Plus 2.0 Arrays. Kaplan Meier/log-rank analysis was used to assess the association between expression of each lncRNA and patients' overall survival. Multivariate cox regression analysis was used to determine if differential expression of lncRNAs were independent predictors of survival. A network based 'guilt-by-association' approach was used to predict the function of lncRNAs associated with patient survival.

Results: Expression levels of 10 lncRNAs in ovarian CAFs were associated with poorer overall survival and 5 were significant by univariate analysis. To adjust for collinearity of the 5 lncRNAs, the first two principal components, response to chemotherapy and debulking status were incorporated into a multivariate model. The first principal component (HR=0.76, P=0.003), response to chemotherapy (HR=2.04, P=0.04) and debulking status (HR=0.27, P=0.03) were independent predictors of survival. Functional enrichment analysis revealed these lncRNAs are likely to play roles in extracellular matrix organisation, immune response, autophagy and cell metabolism.

Conclusions: We identified CAF-derived lncRNAs whose expression levels are associated with survival, suggestive of roles in the tumour-promoting functions of CAFs. A greater understanding of how CAFs are regulated is essential in designing novel therapies targeting the tumour microenvironment.

TS-16, P-27: Progression of colorectal cancer through epidermal growth factor receptor (EGFR)-independent mechanisms

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Epidermal growth factor receptor (EGFR)-targeted therapies have been approved for colorectal cancer (CRC) treatment. However, previous studies have observed that efficacy of anti-EGFR therapy in humans is influenced by the genetic environment on which colonic tumors arise. Mutations in *KRAS* explain some non-responding CRCs, but even in cancers lacking *KRAS* mutations, little is known about which cancers are likely to respond to EGFR targeted treatment. In this study, we used a mouse model with a conditional *Egfr* allele, ($Egfr^{tm1dwt}$ also called $Egfr^{f}$) to demonstrate the existence of EGFR-independent CRCs. We also used a unique mouse model that contains conditionally inactivated *Apc* alleles (Apc^{tm1Tno} or $Apc^{f/f}$) in combination with a conditionally activatable allele of oncogenic *Kras* ($Kras^{tm4Tyj}$ or $Kras^{LSL\text{-}G12D}$) to assess aggressiveness of EGFR-dependent ($Apc^{f/f}$, $Kras^{LSL\text{-}G12D/+}$) versus EGFR-independent ($Egfr^{f/f}$, $Apc^{f/f}$, $Kras^{LSL\text{-}G12D/+}$) tumors. Following delivery of Cre recombinase-expressing adenovirus (AdCre) to the distal colon, 93% of the $Egfr^{f/f}$, $Apc^{f/f}$, $Kras^{LSL\text{-}G12D/+}$ mice develop adenomas. In addition, biweekly colonoscopies confirmed that colonic tumors grow faster in the absence of EGFR. Moreover, RNAseq analysis revealed a group of 32 genes that are differentially expressed in colonic tumors that grow independent of EGFR. Quantitative PCR validated the differential expression levels of several genes involved in IL10RA pathway, including *Sult1a1*, *Il10, Il10ra, Maob, Aadac,* and *Tnf*, in colon tumors without EGFR providing more evidence that EGFR-independent tumors are modulated by the effect of cytokines. These findings demonstrate the existence of an EGFR-independent mechanism by which CRC can progress. This study will advance our understanding of anti-EGFR resistance in CRC treatment, ultimately contributing to more effective therapies.

P-28: A Major Chromosome 5 eQTL Regulates Platelet SERPINE1 (PAI-1) in Inbred Mice

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Plasminogen activator inhibitor-1 (PAI-1, *Serpine1*) is a major circulating inhibitor of fibrinolysis (break down of blood clots). PAI-1 exists in two distinct circulating pools: 10% in the plasma and 90% within platelets. Plasma PAI-1 has been extensively studied, with elevated levels consistently associated with cardiovascular disease. However, the role of platelet PAI-1 (pPAI-1) in cardiovascular disease has not been well studied. Therefore, we investigated pPAI-1 by surveying 10 mouse strains for pPAI-1 antigen levels. We found that the LEWES/EiJ strain had significantly increased pPAI-1 (and *Serpine1* mRNA), compared to C57BL/6J. To identify pPAI-1 regulatory regions, we produced 110 B6LEWESF2 mice, measured pPAI-1 levels and genotyped using the Mouse Universal Genotyping Array (MegaMUGA). QTL analysis identified a major pPAI-1 regulatory locus spanning a 2.4 Mb interval (135.2-137.6 Mb) on Chromosome 5 (LOD score=23.54). Through fine-mapping analysis of three F2 recombinants, we have reduced this interval to 236,441 base pairs. The *Serpine1* gene resides within this candidate interval, strongly suggesting that a cis-eQTL is responsible for pPAI-1 expression differences between LEWES/EiJ and C57BL/6J. Our efforts to fully analyze the candidate interval for putative pPAI-1 regulatory variants are currently limited by an 80,131 base pair gap within this region of the current reference assembly. However, recent sequence finishing efforts have resulted in a C57BL/6J sequence contig (deposited into Genbank) spanning the entire gap. This finished reference sequence should enable the LEWES/EiJ whole genome sequencing reads to be aligned to this region, enabling us to obtain a comprehensive variant list for the pPAI-1 cis-eQTL candidate interval. Identifying pPAI-1 expression control elements will offer insights into platelet-specific gene expression and identify a putative therapeutic target for modulating cardiovascular disease.

TS-2, P-29: Identification and Characterization of Transposable Elements in Genetic Reference Populations

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A large fraction of eukaryotic genomes, including those of mammals, consists of transposable elements (TEs). Spontaneous TE insertions also cause deleterious mutations, and drive chromosome evolution. It is difficult to identify, map, characterize, and determine the zygosity of TEs using current high-throughput short-read sequencing data. Existing approaches search for TEs by aligning billions of mostly irrelevant short reads to either a reference genome or a TE sequence library. These methods are computationally slow, have high false negative rates, and are unable to determine the TE's genomic context and/or zygosity status. Here we present a new msBWT-based TE identification and characterization pipeline that significantly outperforms previous methods in each one of these areas. We apply this method to two different laboratory mouse populations, the Collaborative Cross and a well defined set of commercially available substrains. In each population, we are able to detect fixed, shared and private TEs. We consider private TEs as those whose presence differs between individual samples with identical haplotypes, and these TEs tend to segregate in the relevant population. Thus we conclude that most private TEs represent *de novo* insertion events. We have identified hundreds of private TEs using our approach, we provide preliminary evidence that the number of private TEs depends on genetic background and that private TEs are more deleterious than either shared or fixed TEs. We will discuss the implications of these findings in classical genetic analyses and their impact on rigor and reproducibility.

P-30: The host response to influenza virus infection is highly variable in Collaborative Cross strains

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Influenza virus (IV) infections represent a very serious public health problem. Viral as well as host factors influence the course of an influenza infection. We use mouse genetic reference populations (GRPs) to identify genetic host factors that contribute to resistance or susceptibility to influenza A infections. We have studied the host response of 22 Collaborative Cross (CC) strains after infection with the mouse-adapted influenza virus strain A/HK/01/68 (H3N2). Female as well as male mice were infected intra-nasally with up to three concentrations of H3N2. Body weight and survival were monitored for 14 days. We observed highly divergent susceptibility in the CC strains after influenza infection. The five different *Mx1* (MX dynamin-like GTPase 1) alleles present in the 22 CC strains mainly influenced the outcome of the infection. Nevertheless, we demonstrated that genetic background also influences the course of the infection.
In addition, we characterized the host response in detail in 11 CC strains. RNAseq transcriptional profiling in infected lungs at different time points enabled us to analyze host gene expression and simultaneously monitor virus replication. The amount of viral replication and its time course were different for each strain. Transcriptome studies of the peripheral blood from infected CC mice were used for cross-species comparison. We demonstrate that changes of gene expression after influenza infection in individual genes are highly similar in mice and human blood samples. The top-regulated genes in humans are also differentially regulated in mice. We conclude that the CC strains represent an extremely valuable resource to study the influence of genetic variation on the outcome of influenza infections and thereby to understand individual variations of disease severity in humans.

P-31: Rigorously Free: Mutant Mouse Models at the NIH MMRRC

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The Mutant Mouse Resource & Research Centers (www.mmrrc.org) is the NIH's premier national consortium that provides the worldwide biomedical research community with access to more than 50,000 strains of mutant mice and ES cells. With one of the largest nonprofit repositories of mouse models in the world, the MMRRC distributes and archives models to advance the biomedical research efforts of the scientific research community.

The MMRRC is committed to upholding the highest standards of experimental design and quality control to optimize the reproducibility of research studies using mutant mice. For example, understanding, documenting, and accurately reporting the genetic backgrounds of mouse models used in research is essential for recreating an experimental study and achieving reproducible results.

If research is to be reliable and reproducible, specific quality control and assurance testing need to be in place. This is why mouse models available from the MMRRC repository are annotated with well documented genetic backgrounds. Furthermore, because of our commitment to maintaining specific-pathogen-free vivaria and quality control measures, investigators can rely on mice obtained from the MMRRC to generate robust and reproducible results.

We archive and distribute your mice. We send you the mice you need. It's free.

P-32: Identification of Novel Neuroprotective Loci Modulating Ischemic Stroke

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To identify genetic factors involved in cerebral infarction we have attempted a forward genetic approach using QTL mapping either for cerebral infarct volume after middle cerebral artery occlusion or for pial collateral vessel number, all using common inbred mouse strains. Although in general cerebral infarct volume is inversely correlated with collateral anatomy, we identified several loci that modulate ischemic stroke in a vascular-*independent* manner. To overcome the limited genetic diversity among common domesticated inbred strains, we have expanded the pool of allelic variation by a survey of the parental mouse strains of the Collaborative Cross (CC) that include 3 wild-derived strains. We found that one of wild-derived strains, WSB/EiJ (WSB) breaks general rule that collateral vessel density inversely correlates with infarct volume. This strain and another wild-derived strain, CAST/EiJ (CAST), show the highest collateral vessel densities of any inbred strain we have tested, but infarct volume of WSB is *8.6*-fold larger than CAST/EiJ. QTL mapping between these two strains identified 4 new neuroprotective loci (Chr 1 (5.09 LOD), Chr 6 (7.63 LOD), Chr 13 (6.08 LOD), and Chr 17 (5.57 LOD) modulating cerebral infarct volume while not affecting vascular phenotypes. To identify causative variants in genes mapping within the loci, we surveyed non-synonymous coding SNPs between WSB and CAST and found 194 genes that harbor coding SNP differences in one of the four loci. Further analysis using *in silico* prediction of functional consequences of the amino acid substitutions left only 20 genes in the four intervals. In addition, we performed RNA sequencing to determine strain-specific gene expression differences in brain tissue between WSB and CAST. These results are currently being analyzed. The identification of the genes underlying these loci will provide new understanding of genetic risk factors of ischemic stroke.

TS-15,P-33: Dissecting the role of TLR4 signaling during SARS- and SARS-like CoV infection in mice using the Collaborative Cross reference population and multiple mapping approaches

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Newly emerging pathogens like influenza viruses (H1N1, H5N1, H7N9) and the group 2B coronaviruses (Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), SARS-like CoV HKU3) pose a constant threat to human and animal health leading to significant morbidity and mortality. While it has been shown that the host genetic background plays an important role for the outcome after infection many critical determinants of susceptibility remain to be identified.

The established Collaborative Cross (CC) population of genetically diverse mice and the SARS-CoV as well as HKU3-CoV *in vivo* infection models have been utilized to probe host immunity in the lung. After intra-nasal infection, weight loss, viral titers, as well as lung pathology were evaluated through day four and used to perform quantitative trait locus (QTL) mapping studies.

Among others, multiple candidate genes within the TLR4 signaling pathway were identified: *Anpep* (day four SARS-CoV viral titer QTL – Chr 7); *Git2* (HKU3-CoV mortality QTL – Chr 5); *Wisp1* (day four HKU3-CoV weight loss QTL – Chr 15) and others.

It has been shown that disruption of the TLR3/4 pathway and the TLR-supported balance of the innate immune response renders mice more susceptible to SARS-CoV infection. We were able to show a more resistant phenotype for *Anpep* knock-out (KO) mice as well as a more susceptible phenotype for *Git2* KO mice after SARS-CoV infection compared to wild-type controls. *Wisp1* interacts with a plethora of steps within the TLR4 signaling pathway and the phenotype of *Wisp1* KO mice after SARS-CoV infection remains to be determined.

Using natural allelic variation in mouse models of outbred populations, our data supports the hypothesis that the TLR3/4 signaling pathway plays a pivotal role in regulating the host immune response to multiple group 2B SARS-like CoVs after infection.

TS-7, P-34: Inter-individual variability in epigenetic and genotoxic responses to 1,3-butadiene in a population-based Collaborative Cross mouse model

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1,3-Butadiene (BD) is a known human carcinogen that is both an occupational and environmental health hazard. Although it is well-established that genotoxicity is the key mechanism of BD carcinogenesis, epigenetic events have also been observed. Previous studies in a multi-strain mouse model revealed that inter-strain (e.g. inter-individual) differences exist in both BD-induced DNA damage and epigenetic effects. These studies indicated that variation in epigenetic alterations could drive the inter-individual susceptibility to BD genotoxicity. In the present study, we investigated whether or not there is population variability in epigenetic alterations and genotoxic effects in response to BD exposure by using the Collaborative Cross (CC) mouse model. We tested thehypothesis that there are inter-individual differences in BD-induced epigenetic events and DNA damage. Male mice from 50 CC strains were exposed to 0 or 625 ppm of BD by inhalation (6 hr/day, 5 days/week) for 2 weeks. We evaluated genotoxic and epigenetic effects of BD in tissues that are a target (lung and liver) and non-target (kidney) of BD-induced carcinogenesis. Genotoxicity was assessed by measuring THB-Gua adduct levels. We observed that exposure to BD resulted in variable levels of THB-Gua adducts between strains and tissues. In order to investigate the epigenetic effects, we evaluated the levels of histones H4K20me3, H3K27me3, H3K9me3, H3K9ac, and H3K27ac in the livers. We observed variable responses in a strain-specific manner for all histone modifications as a result of BD exposure. Additionally, we analyzed the status of these histone modifications in the livers of unexposed mice and found that the strains with low levels of THB-Gua adductsafter exposure to BD were characterized by a markedly high histone H3K27ac/H327me3 ratio, a marker of transcriptionally active chromatin. In contrast, this ratio was substantially lower in strains with high levels of THB-Gua adducts.This indicates thatstrain-dependent variability of BD-induced DNA damage and potentially greater tissue susceptibility to carcinogenesis may be predetermined by the pre-exposure epigenome status of a target organ.

P-35: Deletion of mouse *Fmr1nb* gene leads to errors in chromosome alignment and reduced male fertility

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Homologous chromosome pairing, synapsis, recombination and two rounds of the cell division are crucial events inevitable for the success of mammalian meiosis. We use house mouse as a model organism, namely strains C57BL/6J and PWD/Ph, for genetic studies of spermatogenesis and hybrid sterility. A proper course of spermatogenesis is executed and controlled by a large number of specific meiotic factors. Here we demonstrate that FMR1NB protein affects mouse spermatogenesis. Protein coding gene *Fmr1nb* shows high testis-specific expression at the onset and within meiotic prophase I, but its role during meiosis has not been established yet. To address the role of *Fmr1nb* during male germ cell differentiation we have generated the subconsomic-derived null mutant strain C57BL/6J-ChrX.1$^{PWD/Ph}$-*Fmr1nb*- (DX1s.*Fmr1nb*-). We observed that the absence of FMR1NB protein resulted in the reduced sperm count with a significantly higher frequency of malformed sperm heads. Furthermore, sperms lacking FMR1NB exhibited decreased motility and progressive motility compared to control males. Moreover, when DX1s. *Fmr1nb*- males were mated with C57BL/6J females they produced smaller litter size than the DX1s males. Loss of FMR1NB increased apoptosis rate in seminiferous tubules in all meiotic stages. Finally, cytological analysis of spermatocytes revealed higher frequency of XY univalents in metaphase I, which might subsequently lead to higher number of aneuploid sperms and decrease fertility. Presence of metaphase XY univalents was apparently caused by delayed crossing over nodule formation or its absence on the PAR of sex chromosomes during prophase I, which we showed by MLH1 immunostaining. Moreover, we identified the possible reason for that as the expression of Spo11α, a protein responsible for DSBs formation on XY, was significantly diminished in testes of DX1s. *Fmr1nb*- males. Our observations reveal novel functions of *Fmr1nb* in meiosis. However, further investigation is needed to clarify the underlying mechanism.

P-36: A simplified diversity outbred (SDO) mouse population for complex trait analysis

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The Diversity Outbred (DO) population is an outbred population of mice derived from the Collaborative Cross (CC). The DO was envisioned to have a higher density of recombination, and therefore higher mapping resolution for quantitative trait loci (QTL), compared to the CC, due to extended generations of interbreeding. Like the CC, the DO population has genetic contributions from eight founder strains: five 'Castle' strains (A/J, C57BL/6J, 129S1Sv/ImJ, NOD/ShiLtJ, and NZO/H1LtJ) and three 'wild-derived' strains (PWK/PhJ, WSB/EiJ, and CAST/EiJ). Because wild-derived alleles are diluted in the eight-way cross, their detection can be hampered, despite the observation that that majority of the QTL detected to date using the CC or DO are due to one of the wild-derived haplotypes. To overcome this limitation, we derived a new outbred population called the Simplified Diversity Outbred (SDO) population from three wild-derived parental strains of the CC and DO. All possible pairwise crosses among the three strains were performed to produce the G1 founder genetic stock for the SDO. Using the F1 progeny, 40 breeding cages representing a stable stock of 80 chromosomes were established, which is currently at the G14 generation. Sixty-five mice from the G5/G6 generations were phenotyped and genotyped using the GigaMUGA platform. The genotypes were used to evaluate balanced contributions from the founder strains. The same mice were phenotyped for body composition, cholesterol, and hematology for QTL mapping and compared with later generations of the SDO. Results of these analyses show that by reducing the search space to only those alleles with the strongest functional consequences, fewer mice are needed to detect similar QTL's that have been previously mapped in the CC or DO populations. The SDO will be a useful resource for QTL and systems genetic studies because of its reduced genetic complexity in the context of high levels of functional polymorphisms.

P-37: EvalDNA: A machine-learning based tool for the comprehensive evaluation of mammalian genome assembly quality

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Here, we present a novel bioinformatics tool to evaluate the quality of mammalian genome assemblies. Genome assemblies are used widely to guide both wet lab and bioinformatics studies. Accordingly, scientists should choose the highest quality assembly for their organism of interest and be aware of limitations posed by the level of completeness and accuracy of their selected reference assembly. Most current bioinformatics tools that assess assembly quality focus on completeness only, or depend on the availability of another assembly for the organism of interest to evaluate accuracy.

To address this, we developed a tool, EvalDNA (Evaluation of *De Novo* Assemblies), which applies supervised machine-learning methods to combine metrics that reflect the quality of an assembled sequence (e.g. a chromosome) into a single, comprehensive score and does not require an additional genome. EvalDNA first calculates a variety of metrics for a given assembled sequence, including both simple completeness/contiguity metrics and more complex accuracy metrics from existing tools. Next, EvalDNA can either use our mammalian genome-based model (described below), or apply user-provided training data to create a different model, to score the assembly.

Quality metrics from mouse, rat, and human assembly builds, as well as simulated sequences, were used as training data to develop various machine-learning models. Each training chromosome was scored based on alignment against the most recent build of that chromosome to enable supervised learning. The models were assessed using train/test sets, and the best model had an accuracy of ~88.5%. The model's performance on mammalian test data indicates the ability to estimate scores of new assembled mammalian chromosomes. EvalDNA is being used to systematically evaluate several novel assemblies of the *Cricetulus griseus* (Chinese hamster) genome to help establish a better reference genome for *C. griseus* and Chinese hamster ovary (CHO) cells, the preferred platform for therapeutic protein production.

P-38: Network-based prioritization of genes in a large locus: application to spontaneous histamine sensitivity

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Genetic mapping studies typically result in the identification of large quantitative trait loci (QTLs) containing many positional candidate genes. Functionally prioritizing such candidates is a critical step for biological follow up. We have developed a novel *network-based prioritization* method that uses machine learning classifiers to rank positional candidates within QTL based on their participation in functional networks related to the phenotype of interest, and integrates these rankings with positional information from mapping studies. We applied our method to novel mapping data for spontaneous histamine sensitivity (Shs), which in Shs-susceptible SJL/J mice is manifest as death due to hypotensive and hypovolemic shock following histamine challenge of both aged mice and mice exposed to complete Freund's adjuvant (CFA). In this regard, histamine is a mediator of vascular permeability to which some humans are extremely sensitive and plays a role in multiple disease phenotypes, including the rare systemic capillary leak syndrome (SCLS). We show that Shs, controlled by a single recessive locus (*Shs*), maps to Chr 6 within a 55 Mb interval encompassing 1864 protein-coding genes, 109 of which lay within 1 kb of a statistically significant SNP. We used our method to rank among these genes to identify candidates that participate in the functional networks underlying multiple Shs-related phenotypes, including anaphylaxis, Type 1 hypersensitivity, G-protein coupled receptor signaling, and impaired cardiac function. Using our method, we identified *Tril* as the top candidate gene for *Shs*. *Tril* (TLR4 interactor with leucine-rich repeats), encodes for a component of the TLR4 complex that our method predicts is particularly strongly associated with Type 1 hypersensitivity, and known to be activated by CFA. Ongoing work will test the function of this gene as a candidate for *Shs*. These results demonstrate the power of network-based methods to identify strong candidates, even for large loci influencing complex phenotypes.

P-39: One-step generation of conditional knockout mice by CLICK

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CRISPR/Cas9 enables targeted genes in zygotes; however, efficient approaches to create loxP-flanked (floxed) alleles remain elusive. Here, we show that the electroporation of Cas9, two gRNAs, and long single-stranded DNA (lssDNA) into zygotes, termed CLICK (CRISPR with lssDNA inducing conditional knockout alleles), enables the quick generation of floxed alleles in mice and rats. The high efficiency of CLICK provides homozygous knock-ins in oocytes carrying tissue-specific cre, which allows the one-step generation of conditional knockouts in founder (F0) mice.

P-40: *In vivo* analysis of *H19* gene transcriptional mechanism by inversion of the *H19* imprinting control region

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A small subset of autosomal genes in mammals is monoallelically expressed and this unique expression pattern is called "genomic imprinting". Molecular basis of this phenomenon is an allele-specific DNA methylation at the imprinting control regions (ICRs) that is frequently associated with imprinted loci. At the *Igf2/H19* imprinted locus, *Igf2* gene is preferentially expressed from the paternal allele and accelerates embryonic development, while non-coding *H19* RNA is transcribed only from the maternal allele and prevents embryonic overgrowth.

It has been postulated that paternal *H19* gene expression is suppressed by its promoter methylation, which is under the control of the paternal, methylated *H19* ICR. The precise mechanism of the *H19* gene attenuation, however, has not been fully explored. We therefore decided to ask if *H19* promoter methylation was, in fact required for the control of *H19* gene transcription by generating "*H19* ICR-inverted" mice, in which alteration of the promoter methylation level would be anticipated.

The *H19* ICR-inverted locus was generated by CRISPR/Cas9-mediated genome editing. Donor ssDNAs with prospective border sequences after correct inversion, in addition to the Cas9/sgRNA plasmids, were injected into fertilized mouse eggs to facilitate inversion reaction. Out of 27 F0 progenies analyzed, three carried correctly-inverted loci and two independent germ-line transmission lines were established. We present a detailed analysis of the methylation status in the *H19* ICR and promoter regions of the mutant allele, as well as expression level of the *H19* gene in E12.5 embryos, to argue *H19* gene transcriptional mechanism.

P-41: Representation of Orthology in the Alliance of Genome Resources: The Mouse Perspective

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The primary mission of the Alliance of Genome Resources is to develop and maintain sustainable genome information resources that facilitate the use of diverse model organisms in understanding the genetic and genomic basis of human biology, health and disease. Key to the use of model organisms to facilitate our understanding of human biology and disease is the representation of orthology between genes of those organisms. The incorporation of orthology within the Alliance of Genome Resources (www.alliancegenome.org) underpins all aspects of functional and comparative genomics integrated there. The Mouse Genome Database participated in the development of the Alliance orthology resource.

The Alliance methodology for the identification of orthologs among human and Alliance organisms is built on the DRSC Integrative Ortholog Prediction Tool (DIOPT)(https://www.flyrnai.org/cgi-bin/DRSC_orthologs.pl). DIOPT integrates a number of existing approaches including Ensembl Compara, HGNC, Hieranoid, InParanoid, OMA, OrthoFinder, OrthoInspector, PANTHER, PhylomeDB, Roundup, TreeFam, and ZFIN; and assigns a score/count based on these different methods. This method has been extended and modified for use with the Alliance. The core data for the major orthology algorithms is the Reference Proteome Set (ftp://ftp.uniprot.org/pub/databases/uniprot/current_release/knowledgebase/reference_proteomes /Eukaryota/). The orthology is presented in a table on each Alliance gene page where different filters can be deployed allowing users to adjust the stringency of constraints on the orthology set.

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P-42: The Systems Genetics Core Facility at UNC

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The Systems Genetics Core Facility (SGCF) at UNC was established in 2012 to distribute all available strains of the Collaborative Cross (CC) and to provide select genetic data and informatics tools to facilitate the use of CC strains. The CC is a genetic reference population derived from eight inbred strains (including three wild-derived strains). By design the CC population contains high levels of genomewide genetic diversity relative to other mouse resources, and each CC strain has novel combinations of alleles not found in any other mouse inbred strain. These two features combined make the CC population highly variable for many phenotypes of biomedical interest and a rich source of models of human disease. The SGCF is charged with defining the genetic make-up of every distributable CC strain based on dense, robust and public genotyping. To increase rigor and reproducibility of animal model research, the SGCF conducts extensive genetic QC on the CC strains. This includes: a reconstructed founder mosaic of each CC strain from Most Recent Common Ancestors, whole genome sequence from at least one male from each strain, and more recently consensus genotype calls and identification of regions of residual heterozygosity in the current colony through custom genotyping (MiniMUGA).

The SGCF (http://csbio.unc.edu/CCstatus/index.py?run=AvailableLines) has distributed over 16,000 CC mice to >85 laboratories, and currently has 59 CC strains available for distribution to the community. We provide examples of the types of project designs and goals for which the CC are amenable. The SGCF can provide CC breeders (for internal use), experimental cohorts of CC mice, and technical support (such as breeding F2s). Mice, cost estimates, letters of support for grant proposals, and details about technical services are available by contacting Darla Miller at millerdr@med.unc.edu.

TS-8, P-43: Evaluating the transcriptomic plasticity of stem cell quiescence depth with age

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The long-term maintenance of somatic stem cells is in part dependent upon the cellular state of quiescence. In this study we investigate how underlying transcriptional changes in the quiescent program contribute to age-related stem cell inactivation. Taking advantage of the well-defined cyclical periods of stem cell activation and dormancy during the murine hair cycle, pure populations of melanocyte stem cells (McSCs) were isolated from birth, breeding age, and 2-year-old C57BL/6J for whole genome gene expression analysis. The results show global transcriptional reprogramming, as actively dividing stem cell precursors colonize the hair follicle niche and transition into the quiescent state. We first define a core *in vivo* gene expression network of quiescent McSCs, and then evaluate changes in the regulatory mechanisms resulting from the prolonged quiescence experienced by these cells with age. Interestingly aged McSCs predominantly express a reinforced quiescent signature, with an overall reduction in translation and increased expression of genes associated with regulation of homeostasis and adhesion. This suggests that McSC quiescence is not a singular state and, with age, at least a portion of McSCs exist in a deeper level of quiescence. Using *in vitro* quiescent culture conditions, active-, 2- and 4-day quiescent cells were analyzed to confirm how increasing lengths of quiescence in cells of the melanocyte lineage affects their ability to reactivate. We propose, that similarly to what we observe *in vitro,* McSCs become refractory to activation signaling due to the increased depth of quiescence resulting from extended quiescence with age. Informed by our previous transcriptomic analysis we have begun identifying biomarkers associated with changes in depth of quiescence *in vitro* to confirm this process *in vivo*. Our future direction is to further investigate the mechanisms governing deep quiescent stem cell populations and how they can be targeted for tissue regeneration interventions in aged organisms

P-44: Translating *in vitro*-to-*in vivo* phenotypes using genetically-matched organoids and mice from the Collaborative Cross genetic reference population

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Although cardiotoxicity is often the most sensitive parameter for determining threshold exposure levels of toxicants, relatively few environmental chemicals have been tested for cardiotoxicity due to the lack of accurate and high-throughput *in vitro* cardiac models. Translating findings from these current models to human risk assessment is challenging in part due to the lack of genetic diversity represented in these models, as well as limited epidemiological data to validate translation to humans. Animal models have been the gold standard for risk assessment, and in addition to the uncertainty in the accuracy of translation to humans, they are costly, can take many years, and are not amenable to high-throughput screening. While strides have been made in developing *in vitro* models, they have yet to be validated directly *in vivo*. Stem cell research demonstrates that somatic cells of various genetic backgrounds can be successfully reprogrammed to induced pluripotent stem cells (iPSCs), making them an attractive *in vitro* model. iPSCs are being generated from mouse embryonic fibroblasts (MEFs) of individuals from a genetically diverse, mouse reference population, the Collaborative Cross (CC), to model the genetic diversity present in the human population. Cardiomyocytes are differentiated from these iPSCs within the context of a beating embryoid body. These cardiac spheroids and genetically-matched mice from both sexes from eight CC lines are being exposed to a variety of chemicals. Preliminary results already demonstrate inter- and intrastrain variability in a few of these chemicals in the mice. While this project is ongoing, the goal is to create a powerful *in vitro* testing panel that can be validated *in vivo* to model the genetic diversity found within the human population, ultimately allowing more informative risk assessments.

TS-14, P-45: *Msr1* modulates infarct volume through a collateral vessel-independent mechanism

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Infarct volume in experimental stroke models varies across inbred mouse strains. Across most strains, infarct volume is inversely correlated with the extent of collateral vessels, which enable reperfusion of the ischemic territory. However, certain mouse strains share similar collateral vessel anatomy but exhibit significantly different cerebral infarct volumes. In a surgically induced mouse model of ischemic stroke, we previously identified a quantitative trait locus on Chromosome 8 (*Civq4*, LOD = 9.8) that contributes 21% of the phenotypic variance of infarct volume in a cross between C3H/HeJ and C57BL6/J. In this study, we created recombinant congenic mouse lines carrying different segments of the *Civq4* region from C57BL6 introgressed into the C3H background (Line C) and from C3H introgressed into the C57BL6 background (Line B). We examined infarct size pial collateral vessel anatomy in these animals and our results showed that the Line B has a similar number of collateral vessels connections and infarct volume when compared to C57BL6 animals. However, in the group Line C we observed similar number of collateral vessels connections but a larger infarct volume when compared to C3H animals. In addition, a strain-specific nonsynonymous coding variant for *Msr1*, predicted to be damaging, elevated these as candidate genes for *Civq4*. To investigate *Msr1* as candidate gene, we evaluated infarct volume pial collateral vessel anatomy in mouse knockouts for the *Msr1* gene. We observed in *Msr1*-KO animals (C57BL6-$Msr1^{tm1Csk}$) similar number of collateral vessels connections and infarct volume when compared to wild-type C57BL6 animals. However, in *Msr1*-KO animals (C3H background) we observed similar number of collateral vessels connections but a larger infarct volume when compared to wild-type C3H animals. Taken together, our data suggest that the *Msr1* gene might play a role in the neuropathology of cerebral ischemia modulating infarct volume through a collateral vessel-independent (neuroprotective) mechanism.

P-46: MRI-based monitoring of disease progression in a mouse model of KIT-induced cancer to identify effects of genetic background

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Activating mutations of *KIT* play an important role in the genesis and progression of a broad spectrum of neoplastic diseases. In the Munich ENU Mouse Mutagenesis Project we established a mutant mouse line C3HeB/FeJ-*Kit*^{mvd013} - laboratory name MVD013 (mean corpuscular volume down no. 13) carrying an activating *Kit* mutation homolog to the Imatinib®-resistant N822K mutation found in human patients. While these kind of mutations in humans are often associated with highly malignant diseases, mouse models mostly show rather low malignancy. Similar to other mouse models with activating *Kit* mutations heterozygous mutant animals develop erythrocytosis and gastrointestinal stromal tumors (GIST), as well as other types of cancer in advanced age. During backcross of this line to C57BL/6JCrl mice, we observed that in F1-hybrid animals, the timeline of tumor development was changed and neoplasms developed were more malignant in histological appearance. Based on the findings we hypothesize, that malignancy of KIT-induced cancer is influenced by additional unknown genetic features. This impact might be revealed by studying distinct disease progression of varying genetic backgrounds with identical *Kit* mutation. Therefore we propose longitudinal studies of our *Kit* mutation on different genetic backgrounds. Changes in peripheral blood cell counts can be monitored by repeated hematological analysis of blood samples. Intra-abdominal tumor growth and changes in cellular composition of hematopoietic organs like bone marrow and spleen are difficult to follow up noninvasively. Here we report on abdominal *in vivo* magnetic resonance imaging (MRI) as method to follow up age-related disease progression in *Kit* mutant mice compared to age-matched wild-type littermates. Besides abnormal blood cell counts in young mice, our initial MRI findings indicate that altered gastrointestinal morphology and peristalsis belong to the earliest detectable symptoms of the disease.

P-47: The Mouse Gene Expression Database (GXD): fostering insights into the molecular mechanisms of development and disease

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The Gene Expression Database (GXD) is an extensive and freely-available community resource of mouse developmental expression information. Integrating data from RNA in situ hybridization, immunohistochemistry, knock-in reporter, RT-PCR, northern blot, and western blot experiments for wild-type and mutant mice, GXD currently holds over 1.6 million annotated expression results from more than 14,700 genes and includes almost 340,000 expression images. Data are obtained from the scientific literature and by collaborations with large-scale data providers. GXD curators annotate expression data in standardized ways using genetic nomenclature, controlled vocabularies, and an extensive anatomical ontology. Because GXD is an integral part of the larger Mouse Genome Informatics (MGI) resource (www.informatics.jax.org), this curation work results in a deep integration of mouse expression, genotype, and phenotype data that, in turn, provides researchers with extensive search capabilities. We have now developed several new interface utilities that allow the direct correlation of expression and phenotype data and thus foster insights into the molecular mechanisms of development and disease. A new Gene Expression + Phenotype Comparison Matrix visually juxtaposes tissues where a gene is normally expressed against tissues where mutations in that gene cause abnormalities. The anatomy axis of the view can be expanded and collapsed, allowing users to interactively explore correlations between gene expression and phenotype at different levels of detail. The Mouse Developmental Anatomy Browser now provides not only access to expression data for a given anatomical structure (as before), but also to corresponding phenotype data. Conversely, MGI's Mammalian Phenotype Browser now points to tissues affected by a given phenotype that in turn link to the wild-type expression data for these tissues. Improvements have also been made to the GXD Differential Expression Search. Visit the GXD Home Page at www.informatics.jax.org/expression.shtml to explore GXD. GXD is supported by NIH/NICHD grant HD062499.

TS-9, P-48: Discovering Genetic Modifiers in a new Mouse Model of Niemann-Pick Disease, Type C

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Niemann-Pick Disease, Type C (NPC) is a fatal neurodegenerative disorder that exhibits intracellular accumulation of unesterified cholesterol in late endosomes/lysosomes and marked accumulation of glycosphingolipids in neuronal tissue. These subcellular pathologies eventually lead to phenotypes of hepatosplenomegaly and neurological degeneration leading to premature death. NPC disease is extremely heterogeneous in the timing of clinical presentation (prenatal to adulthood), is associated with a wide spectrum of causative *NPC1* mutations, and the time of onset or severity of the disease shows little concordance with the predicted consequences of *NPC1* gene mutation on protein function. Currently there are no FDA-approved therapies that effectively increase lifespan or slow disease progression.

To further explore the influence of genetic background in NPC1 severity and mapping potential genetic modifier(s), we generated a mouse model for NPC harboring a novel allele ($Npc1^{em1Pav}$ aka $Npc1^{S1062_I1064del}$) using CRISPR/Cas9 on a C57BL/6J background. By using speed congenics we established N4 intercross mutants with BALB/cJ with a significant ($p<0.0001$) increased lifespan (84.3 + 5.25 days) compared with the original C57BL/6J colony (69.7 + 4.36). Backcross N2 $Npc1^{S1062_I1064del}$ homozygous mutants also showed a significant increase in lifespan (78.36 + 6.90) with greater variance suggesting strain-specific modifiers influencing NPC1 disease severity.

Using the GigaMUGA genotyping array we analyzed N2 mutants (N=202) and detected significant linkage to markers on Chromosome 1 (LOD=5.57) and Chromosome 7 (LOD=8.91). To follow up on these results we are currently integrating linkage data, RNA seq and WES data from an NPC1 patient cohort with the hopes of prioritizing candidate genes for *in vitro* and *in vivo* validation. Identification of modifiers will contribute to our understanding of the highly variable phenotype observed in NPC patients and advance our efforts to improve patient diagnosis and therapy.

P-49: High-throughput mouse genotyping by next generation sequencing

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Wellcome Sanger Institute

The Sanger Mouse Core team provides genotyping services for external collaborative projects and our Institute Faculty teams. Alleles including CRISPR-mediated point mutations, exon deletions and traditional gene targeting events (such as the EUCOMM/KOMP collection) are all routinely processed using a variety of methods.

For the EUCOMM/KOMP alleles, the presence of the selection cassette allowed us to perform high-throughput screening in a gene-independent manner. With CRISPR-mediated mutations in zygotes, however, the selection cassette is not present, and universal genotyping is not applicable. Gene-specific qPCR and end-point gel-based PCR all provide solutions, and have their own advantages and disadvantages when it comes to assay design. We have therefore begun investigating genotyping mice by next generation sequencing (NGS) as a potential alternative.

By multiplexing the WT and mutant-specific reactions, the genotype can be called by the relative sequence counts of each product. Assays are designed to be as close in size as possible to each other to avoid biasing amplification of one allele over another.

One potential problem with any assay design is finding unique areas of the genome when dealing with gene families or areas with high homology to other regions. These can result in misleading or ambiguous genotypes for either qPCR or end point assays. Here we show that genotyping by NGS can negate these issues by simple, automated filtering of undesired sequences. Analysis and genotype calls can also be fully automated, using FASTQ or FASTA input files and an in-house perl script and SQL database.

P-50: Big data in mouse phenotyping: Discovery of regulatory genomic patterns

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The value of any large-scale genetic phenotyping program, such as the International Mouse Phenotyping Consortium, significantly increases with the ability to detect and cluster shared gene regulation elements. However, the considerable number of genes in large-scale phenotyping and the frequently unkown function of the genes render the assessment of shared gene regulation elements very challenging. We demonstrated an only genomic pattern driven approach to predict candidates for transcriptionally co-regulated genes that exhibit same phenotypes, without the need of prior annotation of the function of a gene. To this end, we did a gene set enrichment analysis using so-called Multiple Organized Regulatory Elements (MOREs), which are regulatory genomic patterns in promoter regions composed of two to six transcription factor binding sides arranged in a specific order. For the analysis we used commercially available software from Genomatix. In a pilot project we analyzed knockout genes with strong phenotypes and identified with a defined workflow common regulatory elements in the promoter regions of these genes. These results were used to predict further candidate genes that might be associated to a specific phenotype. Despite this success, this approach proved to be time and labor intensive. The demand for efficient big data analyses requires automation of this workflow to enable exhaustive analyses rather than the greedy analyses performed in our pilot study that had required manual priorization. Furthermore, it will allow us to replace arbitrary thresholds by data-derived thresholds. All this will lead to a robust, efficient and universal large-scale approach to discover regulatory genomic patterns.

P-51: Mechanisms of bacterial tolerance in the Collaborative Cross genetic reference mouse population

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There is growing appreciation that some infected hosts can be "tolerant" to an infectious agent, which is defined as the ability to tolerate colonization by a pathogen while maintaining host health. While this phenotype has been studied in plants, little is known about biological mechanisms of tolerance in mammals. To investigate genetic components of tolerance, we are using the Collaborative Cross (CC) genetic reference mouse population to identify CC lines that are tolerant to infection with *Salmonella enterica* serotype Typhimurium (STm). Three males and three females are infected per CC line, and mice are continuously monitored for activity, body temperature, and health status over a seven-day infection. After euthanasia, bacterial load is determined from the intestine as well as from systemic organs including liver and spleen. Preliminary screening of twenty-six CC lines has revealed a wide range of responses, with five lines categorized as sensitive, twelve lines as resistant, and nine lines showing indications of tolerance. Preliminary quantitative trait loci (QTL) analysis reveals genetic loci likely responsible for differential response to infection, including body weight change, survival time, and bacterial load in each organ. Some QTL are organ or phenotype specific, while others like a bacterial load QTL are shared across organs, making this an attractive candidate for tolerance. In addition, tissue pathology, immune markers in serum and blood, microbiome composition, and transcriptomics have been generated to elucidate causative loci underlying the QTL. The nine candidate tolerant lines are being further characterized over a twenty-one-day post-infection period. Integration of these data will be used to reveal genetic and molecular mechanisms of tolerance to STm infection, potentially revealing host-targeted interventions for susceptible populations.

P-52: Nano-trapping B Cell Chemoattractant CXCL13 Reduces Bregs in Tumor Microenvironment and Inhibits Desmoplastic Tumors in Murine Orthotopic Models

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Tumor growth depends on the interactions between multiple cell types in the tumor microenvironment (TME). In this study, we highlight the interleukin 10 (IL-10) producing regulatory B cells (Bregs) ($IL\text{-}10^{+}CD1d^{+}CD5^{+}CD138^{+}$) as a new key element in controlling the immunosuppressive TME in different desmoplastic murine tumor models. The C-X-C Motif Chemokine Ligand 13 (CXCL13), which is produced mostly by tumor-associated fibroblasts (TAFs), recruits B cells to the TME, where they differentiate into Bregs. Bregs promote tumor progression by attenuating anti-tumor immunity via the T-cell immune response suppression through the secretion of anti-inflammatory mediators, such as IL-10, TGF-β and IL-35, and by facilitating the generation of Tregs. These Bregs can suppress the anti-tumor responses of natural killer (NK) cells, as well as the local activation of dendritic cells (DCs).

We identify the preferential accumulation of a Breg cell subset with regulatory activity localized within the TME in murine pancreatic cancer, BRAF-mutant melanoma, and triple-negative breast cancer. Herein, we developed an innovative anti-cancer strategy focused on reducing Bregs differentiation and accumulation in tumor microenvironment. hence reducing tumor progression. Toward this aim, we have designed an affinity protein, which we call a trap, that binds with CXCL13 with high affinity in a manner similar to a monoclonal antibody and blocks its biological function. We used nanoparticles to deliver and express a plasmid DNA encoding the CXCL13 trap in several genetic modified mice tumor model to test the hypothesis and therapeutic efficiency.

The results indicated that, when effectively delivered to the tumor, the CXCL13 trap reduced Bregs in the TME, dramatically stopped the tumor growth in pancreatic tumor, shrank the advanced BRAF-mutant melanoma, and inhibited triple-negative breast cancer growth. The affected tumor remained dormant long after dosing stopped, resulting in a prolonged progression-free survival of the host.

TS-10, P-53: The Loss-of-Function Intolerant *Actr2* Gene is Resistant to Gene Targeting and Genome Editing

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Susceptibility to venous thromboembolism (VTE) is increased by inheritance of Factor V Leiden ($F5^{L}$), however, only ~10% of people inheriting $F5^{L}$ develop VTE, illuminating the need to identify genes that modify VTE risk. We recently reported a dominant, p.R258G missense mutation in the *Actr2* gene ($Actr2^{MF5L12}$) as a major thrombosuppressor of perinatal lethal thrombosis in mice homozygous for $F5^{L}$ ($F5^{tm2Dgi}$) and heterozygous for tissue factor pathway inhibitor ($Tfpi^{tm1Gjb}$). The *Actr2* gene encodes ARP2 actin-related protein 2, which is an essential member of the Arp2/3 complex. Arp2/3 is ubiquitously expressed and is necessary for actin cytoskeletal regulation in all eukaryotic cells, including platelets. We recently attempted to generate independent R258G missense and knockout alleles in mice using CRISPR/Cas9 genome editing. The failure to produce any mice carrying the *Actr2-* allele suggested that even heterozygosity is incompatible with embryonic survival. To further investigate this unexpected finding, ES cells (Clone:EPD0727_2_H12) containing the targeted $Actr2^{tm1a(KOMP)Wtsi}$ "Knockout First" allele were obtained. Transfer of 79 injected blastocysts into foster mothers yielded five males with 20-90% chimerism. Successful germline transmission occurred from only the 20% chimera. *Actr2* locus targeting was confirmed by 5' and 3' long range PCR from tail DNA in the four non-transmitting chimeras. However, long range PCR analysis of the 20% chimera and its progeny revealed only mistargeting outside of the *Actr2* locus. This demonstrates that the KOMP ES cells were heterogeneous for correct/incorrect *Actr2* targeting and confirms that a 50% reduction of *Actr2* function is incompatible with survival. In summary, our attempts to generate *Actr2-* mice illustrate the importance of meticulously screening for correct/incorrect vector incorporation in gene targeting experiments. This is especially relevant for loss-of-function intolerant genes such as *Actr2*, because targeting this subset of genes results in negative selection.

TS-13, P-54: Variable effects of steroid treatment on patient derived keloid fibroblasts – is underlying genetic/epigenetic heterogeneity the cause?

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Keloid lesions are fibrotic scars on the skin that occur in susceptible individuals due to abnormal wound healing and are characterized by excessive collagen production. Certain populations, such as African Americans and Asians have a much higher incidence of keloids, suggesting a strong genetic or epigenetic component that contributes to this disease. Due to their high recurrence rates following surgical excision, keloids are one of the most challenging dermatological conditions to effectively treat. Although there is no universally accepted treatment for keloids, intralesional corticosteroids are commonly used as first-line therapy to aid in softening and flattening keloids by diminishing collagen synthesis and inhibiting keloid fibroblast proliferation. Corticosteroids are also used as adjunctive therapy to surgical excision and have demonstrated a reduction in recurrence rates. However, some reports in the literature suggest that a significant number of keloid patients may be refractory to steroid therapy, with some patients exhibiting a worsening of their keloids following steroid therapy. Remarkably, these findings have been replicated *in vitro* in our laboratory using patient derived keloid fibroblasts that exhibit highly variable cell proliferation rates upon steroid treatment. Some keloid fibroblasts are sensitive to steroid treatment and exhibit attenuated proliferation, while others are either refractory or show hyperproliferation upon steroid treatment. We are now interested in uncovering the underlying molecular mechanisms that may mediate the variable effects of steroid treatment on keloids from different patients. Based on our data so far, we hypothesize that genetic and/or epigenetic heterogeneity may lead to the variable responses observed upon keloid therapy using steroids. We are currently testing our hypothesis by systematically evaluating DNA methylation and post-translational histone modifications in keloid derived fibroblasts as well as employing transcriptomic and whole genome sequencing approaches to study the underlying differences between keloid fibroblasts that are either sensitive or resistant to steroids.

P-55: A *Sox10* Multi-Spectral allele for *in vivo* Ca^{2+} imaging of neural crest lineages

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Sox10 is a transcription factor of the Sry-like HMG domain family that is essential for the development of neural crest cell lineages within the central and peripheral nervous systems. Within neural crest populations, transient fluxes of calcium have been shown to effect cell migration, communication, and differentiation. To observe this phenomenon directly in neural crest progenitors via live cell imaging, we have generated a multi-spectral (MS) allele of *Sox10* that encodes three separate fluorescent reporters. Our construct includes a membrane bound reporter (Myr-Apple) to visualize the cellular membrane, a nuclear reporter (H2B-mCer3) to localize the nucleus, and a genetically encoded calcium indicator (GCaMP5) to monitor calcium flux during cell signaling. This new allele is designated Tg(Sox10-GCaMP5,-HIST2H2BE/mCer3,-Apple*)1Sout or called "*Sox10*-MS" for short. The *Sox10*-MS construct relies upon long-range regulatory sequences in a *Sox10* BAC to drive transgene expression and avoid disruption of the endogenous *Sox10* locus. As a result, *Sox10*-MS mice are phenotypically normal. Our initial characterization of this new reporter demonstrates that the transgene recapitulates endogenous *Sox10* gene expression in migrating enteric progenitors in the fetal intestine as well as in adult enteric glia. Thus, this *Sox10*-MS allele enables studies of cell migration and communication in the context of normal neural crest development, maturation, and enteric glial physiology. Homozygous *Sox10*-MS mice are also viable, indicating that the transgene insertion site is innocuous. The *Sox10*-MS single allele signaling reporter is advantageous because it permits ready coupling with other mutant lines for analysis of defects in neural crest development and alterations in glial signaling due to disease pathology.

P-56: GUDMAP: An integrated Resource for GenitoUrinary Development

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The GenitoUrinary Development Molecular Anatomy Project (GUDMAP) is an open access online resource developed by a consortium of laboratories (the PIs listed here) working to provide the scientific and medical community with gene and protein expression data, transgenic mice, high-resolution 2D and 3D morphological and molecular data, as well as tools to facilitate research and teaching in genitourinary (GU) development and related diseases. The goal is to stimulate further research into genitourinary development by identifying novel cell types and gene expression domains in the kidneys, ureters, bladder, prostate, urethra, external genitalia, and associated reproductive structures and accompanying innervation. The GUDMAP database includes murine in situ hybridization, immunofluorescence, 3-D Optical Projection Tomography (OPT) and nanoscale computed tomography (nanoCT) images, microarray, RNA-Seq, and ChIP-Seq gene expression data that is complemented by high-resolution histology. Expression data are annotated using a high-resolution ontology specific to the developing murine GU system. The database is searchable based on gene name, function, or anatomical structure. Database queries can be refined based on stage, organ, or expression. Recent efforts have integrated complementary gene expression and morphological data on human GU and reproductive tract development into GUDMAP to facilitate comparative studies. Online tutorials are provided that detail the complex anatomical changes that occur during GU development. GUDMAP data are curated and freely accessible at www.gudmap.org. GUDMAP is supported by the NIDDK.

P-57: Gut microbial profiling of a new mouse mutant that is resistant to diet induced obesity

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BACKGROUND: Diet can shape the gut microbial community of both humans and mice. A new mouse mutant C57BL/6J-*hlb444* (HLB444) identified by elevated plasma triglycerides was found resistant to obesity when fed a high-fat (HF) diet. We profiled the microbiome of HLB444 & control animals on chow diet and after shift to HF.

METHODS**:** Fecal pellets were collected from C57BL/6J (B6) controls and HLB444 fed standard chow. Pellets were then collected 24, 48, and 72 hours and five weeks after introducing a HF diet containing 45% kcal from fat (Envigo™ TD.08811). 16S rRNA V1-V3 was sequenced and whole metagenome shotgun sequence data and metatranscriptome data were obtained using Illumina NexteraXT and ScriptSeq Complete Gold Epidemiology libraries, respectively. Assembled metagenome and metatranscriptome data were integrated to define differentially expressed genes.

RESULTS**:** Because reference databases for mouse gut microbial species are limited, we performed *de novo* assembly, yielding >750 Mb of assembled sequence and >700,000 predicted open reading frames. Control and mutant animals showed marked differences in microbial community composition on chow. On HF, both genotypes demonstrated rapid alterations in community structure. Gut microbial communities in HLB444 and B6 differed in response to HF, with B6 exhibiting loss of *Anaeroplasma* and HLB444 exhibiting reduced *Akkermansia*. The Bacteriodetes to Firmicutes ratio declined in both genotypes, with greater reduction in HLB444. On HF, HLB444 animals gained little body fat, had smaller adipocytes and did not develop liver steatosis.

CONCLUSION: The differential response of HLB444 to HF diet provides insight into the role of the microbiome in adapting to dietary change. Differences in microbial composition on chow and with HF diet between control and HLB444 will add to understanding how dietary manipulations impact gut microbiota and help describe development of obesity towards finding strategies to ameliorate obesity and its comorbidities.

P-58: Pipeline for quality control of large-scale mouse phenotype data

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The International Mouse Phenotyping Consortium (IMPC, www.mousephenotype.org) has generated multi-dimensional phenotyping data for over 6,000 mouse knockout lines, from 12 centres worldwide. The phenotyping pipeline covers a broad spectrum of tests performed from weeks 9 to 16, and is currently expanding to include tests performed around 52 weeks of age. This results in large quantities of diverse phenotyping data that require quality control (QC) prior to identification of phenotypes, which are made public via the IMPC portal.

QC is performed by a team of data wranglers who explore the data to identify potential issues. These issues are reported to the phenotyping centres for their review and subsequent fixes of data where an error is clear.

With increasing amounts of data being generated, more accurate and automated methods for QC are required. As a result, an automated pipeline, which uses both knowledge about the phenotyping procedures and information gained from previously raised QC issues, has been developed. For each parameter:

- Step 1. Identify impossible measurements using knowledge of procedures

- Step 2. Determine if maximum and minimum QC thresholds can be set using information garnered from previously resolved issues

- Step 3. If there is little information in resolved QC issues, apply a random forest model trained on previously raised QC issues, both resolved and unresolved, to predict which numeric measurements are likely to be issues

- Step 4. For numeric parameters where there is little or no previous information to set QC boundaries, potential issues are identified by selecting all measurements less than or greater than two standard deviations from the baseline mean

This pipeline has allowed for a much reduced set of potential QC issues requiring a wrangler's review, without losing the ability to accurately identify issues, resulting in a saving in time vital for the ever-expanding nature of the IMPC dataset.

P-59: Abnormal embryonic spacing in C3HeB/FeJ mice is under complex genetic control

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Embryonic spacing, the positioning of embryos prior to implantation, is normally equidistant between embryos within the uterus. This preimplantation process is tightly regulated and important for healthy pregnancies in multiparous species including swine, sheep, goats, rabbits, mice, and rats. Additionally, embryonic spacing and migration is thought to play a significant role in early pregnancy of non-polytocous species, such as humans. Abnormalities in spacing and migration can result in growth restriction and abortion/reabsorption. We discovered that mice from C3H lines have abnormal embryonic spacing. To elucidate genetic mechanisms associated with embryonic spacing, C57BL/6J, C3HeB/FeJ, and C3H/HeJ strains were analyzed. C57BL/6J have evenly spaced embryos, whereas both C3H sublines have unevenly spaced embryos with frequent embryonic crowding and fused placentae. Numerous physiological mechanisms were tested and determined not to contribute to the C3H phenotype, including sex of the embryo, parity, and timing of implantation. Pedigree analysis suggested that both maternal and embryonic genetic factors are responsible. Additionally, quantitative trait loci analysis using 12 BXH recombinant inbred lines, generated from C57BL/6J and C3H/HeJ was performed. These data implicate several genomic regions containing candidate genes poly ADP-ribose polymerase family member 8 (*Parp8*) and embigin (*Emb*).

P-60: Unbiased *in vivo* CRISPR screen to identify novel T-cell Immune Checkpoints for cancer immunotherapy

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Harnessing the anti-tumour potential of T-cells for cancer treatment has been successful for a number of malignancies. The blockade of immune checkpoints, such as the T-cell receptor PD-1, reduces tumour burden in patients. Such treatment efficiency is further improved when PD-1 is targeted simultaneously with other known checkpoints such as CTLA4 or LAG3. Despite the potential of co-targeting multiple checkpoints for future therapeutics, the combinatorial co-inhibition of all known as well as yet undiscovered immune checkpoints has not been systematically assessed. We aim to map the combinatorial landscape of known checkpoints coupled with PD-1 blockade as well as discovering potentially new targets for immune activation against cancer. In order to screen which combinations enable T-cell infiltration and tumour reduction, we will transplant hematopoietic stem cells carrying a targeted library of CRISPR/Cas9 induced mutations into a PD-1 suppressed mouse melanoma model.

To establish this screening protocol, we first carried out a proof-of-principle experiment targeting the locus of known checkpoint PD-1 (*Pdcd1*). After electroporating anti-*Pdcd1* gRNAs into lineage depleted bone marrow cells, the successfully electroporated cells were sorted and transplanted to sub-lethally irradiated mice, 50,000 cells per animal. Transplantation was confirmed by allotype-based sorting of blood cells 4 and 8 weeks after the injections. At 10 weeks, 50,000 B16 melanoma cells were injected to the flank of the edited mice. In the setting of *Pdcd1* mutant immune cells, the tumour growth was visibly repressed within the first two weeks compared to the mice with wildtype cells. We will next apply this protocol to a library of gRNAs.

P-61: Murine strain differences in hallmark responses to the air pollutant ozone

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Ambient ozone, a common air pollutant, has significant impacts on public health with effects ranging from airway inflammation and oxidative stress to exacerbation of existing lung disease. Studies in both humans and rodents have established that responses to ozone, including neutrophilic inflammation, lung injury, and lung function, are characterized by high inter-individual and low intra-individual variation, suggesting a role for natural genetic variability. Unbiased, genome-wide analyses would provide important insights into the genetic determinants of response to ozone; however, these studies are not feasible in humans. Inbred mouse strains, such as the Collaborative Cross (CC) genetic reference population, represent an alternative approach. We exposed 6-week-old, female mice from 13 CC strains to 2 ppm ozone for 3 hours. Twenty-one hours thereafter, mice were sacrificed and bronchoalveolar lavage (BAL) was performed. Two hallmark responses to ozone exposure were measured: neutrophilic accumulation in the lungs and total protein recovered by BAL. The main effect of strain was analyzed using one-way analysis of variance (ANOVA) and revealed a statistically significant effect for percentage neutrophils ($p = 7.0 \times 10^{-3}$), neutrophil counts ($p = 3.586 \times 10^{-5}$), and total protein ($p = 2.323 \times 10^{-7}$) in BAL. Notably, some CC strains display more extreme responses than a commonly used inbred strain (C57BL/6J), and these responses are not necessarily correlated. Consistent with previous studies, our initial strain survey demonstrates that genetic background influences hallmark responses to ozone exposure and suggests that the CC is a valuable resource for uncovering genetic modifiers of ozone responses. Future studies will extend this approach to all available CC strains and incorporate whole-transcriptome profiling of alveolar macrophages, a critical resident immune cell population, in order to assess their contributions to the response to ozone exposure.

P-62: Imprint setting mechanisms at the human Angelman/Prader-Willi Locus in BAC transgenic mouse

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Genetically imprinted domains are defined as those with differential gene expression of paternal and maternal alleles. Angelman syndrome (AS) and Prader-Willi syndrome (PWS) are two clinically distinct genetic disorders which cause lifelong disability. Both PWS and AS are caused by changes in the function of the same genetically-imprinted domain located in human chromosome 15q11-13. Similar to other imprinted domains, the PWS-AS locus is regulated by an imprinting center (IC), which controls this allele-specific expression. The IC for this locus is defined of two distinct elements that are deleted in some individuals with imprinting defects. The PWS-IC functions in somatic tissue to activate expression of paternally expressed genes. The AS-IC epigenetically inactivates the PWS-IC in oocytes thereby preventing PWS-IC function on the future maternal allele. Our long-term goal is to understand the specific molecular mechanisms by which the human AS-IC initiates maternal allele identity. Although the mouse and human AS-IC lack DNA sequence homology, our lab has demonstrated that in mouse and human, AS-IC function consists of being an oocyte specific transcriptional promoter that drives transcripts across the PWS-IC which leads to epigenetic inactivation of PWS-IC. The goal of this research is to develop an *in vivo* system to analyze the human AS-IC element. Thus, I will create a series of transgenic mouse lines containing a large part of the PWS-AS human locus.

P-63: CRISPR off-target analysis in genetically engineered rats and mice

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Despite widespread use of CRISPR, comprehensive data on the frequency and impact of Cas9-mediated off-targets in modified rodents are limited. We generated deep-sequencing data from 81 genome-editing projects on mouse and rat genomes at 1,423 predicted off-target sites and confirmed editing at 32 off-target sites. We show that high-fidelity Cas9 versions reduce off-target mutation rates *in vivo*. Using whole-genome sequencing data from ten mouse embryos treated with a *Pnpla3* single guide RNA (sgRNA), and from their genetic parents, we found a total of 43 off-targets, 30 of which were predicted by an adapted version of GUIDE-seq. As this data was recently published (Nat Methods 2018: 15(7):512-514), we here discuss how we have implemented our findings into our own workflow and provide recommendations for the use of CRISPR in rodent models.

TS-4, P-64: Genomic signatures of age-dependent hybrid male sterility in the mouse

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Hybrid male sterility (HMS) is a reproductive barrier that restricts gene flow between two subspecies of mice, *Mus musculus musculus* and *M. m. domesticus*. Two major loci have been previously linked to HMS in laboratory crosses, but we observed wide variation in fertility and reproductive traits among hybrids with identical genotypes at those loci. We characterized reproductive trait variation in a panel of hybrid males bred by crossing musculus-dervied PWK/PhJ strain females to males from four inbred mouse strains of primarily *domesticus* origin. These hybrids displayed three distinct trajectories of fertility: complete sterility, complete fertility, and age-dependent fertility. Males that displayed age-dependent HMS were fertile between 15-35 weeks of age with moderate penetrance. These results point to multiple segregating HMS modifier alleles, some of which have an age-dependent mode of action. Whole-testis gene expression patterns distinguished the three fertility trajectories and implicated key regulatory pathways involved in changes to fertility with age. A subset of genes also displayed differences in allelic bias between types of hybrids. Allele-specific gene expression could inform the molecular mechanisms of HMS in mice.

P-65: The New and Improved CrePortal: a Comprehensive, Annotated Mouse Genome Informatics Resource of Recombinase-Expressing Mouse Lines

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Over 20,000 conditional-ready mice have been generated since the introduction of conditional mutagenesis technology in the early 1990s. The technology is based on mice having a pair of recombinase recognition sites flanking the targeted genomic region and also expressing a site-specific recombinase (such as cre, flp, dre, or phiC31). Conditionally targeted mice are created by crossing mice carrying conditional-ready alleles with mice carrying recombinase expressing alleles. Since mutation is controlled by the spatio-temporal specificity of the recombinase expression, driven by a driver gene enhancer or promoter, it is crucial to characterize its expression pattern as fully as possible. A large volume of published results on recombinase activities at the intended locations and time points exists. However, negative results or unintended activities are often not reported. The CrePortal (informatics.jax.org/home/recombinase), the latest version of which we present here, catalogs published and unpublished researcher-submitted recombinase driver mouse lines to facilitate identification of the most suitable lines for conditional mutagenesis. This centralized, comprehensive, well-annotated dataset currently provides data for almost 3000 transgenes and knock-in recombinase driver alleles. Recombinase activity is annotated not only regarding its reported activity, but also regarding where activity is not detected. In the current release, users can search for activities detected in specific anatomical structures and nowhere else. Recombinase allele summary pages display a link to new matrix views of driver gene expression and recombinase activity data by tissue, allowing users to easily compare endogenous gene expression with reported activities of all recombinases driven by the same driver of expression. The matrix views are available to view from cre search summary and detail pages, and from gene detail pages.

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P-66: Correlation of Cas9 expression and the mutation pattern in mouse embryos

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Genome editing technologies enabled us for a high-throughput, high-efficiency and low-cost production of genetically modified animals. We have established many knockout and knock-in mice and rats with CRISPR-Cas9. However, mosaic mutations or no targeted mutations can be detected at several target sites, which makes difficult to establish the mutant strains in mice and rats. In this study, we visualized sequential expression of Cas9 with P2A peptide and GFP protein in mouse embryos and analyzed the relationship between the Cas9 expression level and Cas9-induced mutations at *Tyr* gene. Fluorescence observation in mouse embryos at 2-cell stage revealed that Cas9 expression level were widely varied even in the same experiment. Genetic analysis showed that there was no correlation between knockout efficiency at *Tyr* gene and Cas9 expression pattern, while embryos highly expressing Cas9 significantly carried homozygous mutation at the target site. These results suggested that a visualization of Cas9 expression can be a good selection marker for homozygously mutated embryos before the transplantation.

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Laura Hergott	The Hospital For Sick Children	Toronto, Canada
Viive Howell	University of Sydney	St Leonards, Australia

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Samir Kelada	University of North Carolina at Chapel Hill	Chapel Hill, NC USA
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Heike Kollmus	Helmholtz Centre for Infection Research	Braunschweig, Deutschland
Markus Kraiger	Institute of Experimental Genetics	Munich, Germany
Dimitry Krementsov	University of Vermont	Burlington, Vermont USA
William Law	Johns Hopkins School of Medicine	Baltimore, MD USA
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Sarah Leist	University of North Carolina at Chapel Hill	Chapel Hill, NC USA
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Hitomi Matsuzaki	University of Tsukuba	Tsukuba, Japan
Monica McAndrews	The Jackson Laboratory	Bar Harbor, ME USA
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Kelsey Noll	University of North Carolina-Chapel Hill	Chapel Hill, NC USA
Nate Nowak	Transnetyx	Cordova, TN USA
Joseph Palmer	University of Alabama, Birmingham	Birmingham, AL USA
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Julia Popp	Texas A&M	College Station, Texas USA
Andrew Pospisilik	Max Planck Institute of Immunobiology and Epigenetics and Van Andel Institute, Grand Rapids	Freiburg, Germany
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Jesse Raab	University of North Carolina-Chapel Hill	Chapel Hill, NC USA
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Xin Rairdan	Genentech	San Francisco, CA USA
Birgit Rathkolb	Ludwig-Maximilians-University	Munich, Germany
Laura Reinholdt	The Jackson Laboratory	Bar Harbor, Maine USA
Martin Ringwald	The Jackson Laboratory	Bar Harbor, ME USA

Jorge Rodriguez-Gil	NIH	Bethesda , USA
Merone Roose-Girma	Genentech	South San Francisco, CA USA
Nadia Rosenthal	The Jackson Laboratory	Bar Harbor, ME USA
Edward Ryder	Wellcome Sanger Institute	Hinxton, United Kingdom
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Kristin Scoggin	Texas A&M University	College Station, TX USA
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John Shorter	University of North Carolina- Chapel Hill	Chapel Hill, NC USA
Amy Siebert-McKenzie	Oakland University	Rochester, MI USA
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Cynthia Smith	The Jackson Laboratory	Bar Harbor, ME USA
Jennifer Smith	Medical College of Wisconsin	Milwaukee, WI USA
Yuna Son	Florida State University	Tallahassee, FL USA
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Karen Svenson	The Jackson Laboratory	Bar Harbor, Maine USA
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Keiji Tanimoto	University of Tsukuba	Tsukuba, Japan
Lisa Tarantino	University of North Carolina- Chapel Hill	Chapel Hill, NC USA
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Deborah Threadgill	Texas A&M University	College Station, Texas USA
Rebekah Tillotson	The Hospital for Sick Children	Toronto, Canada
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Anna Tyler	The Jackson Laboratory	Bar Harbor, ME USA
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Neeti Vashi	Hospital for Sick Children, Toronto	Toronto, Canada
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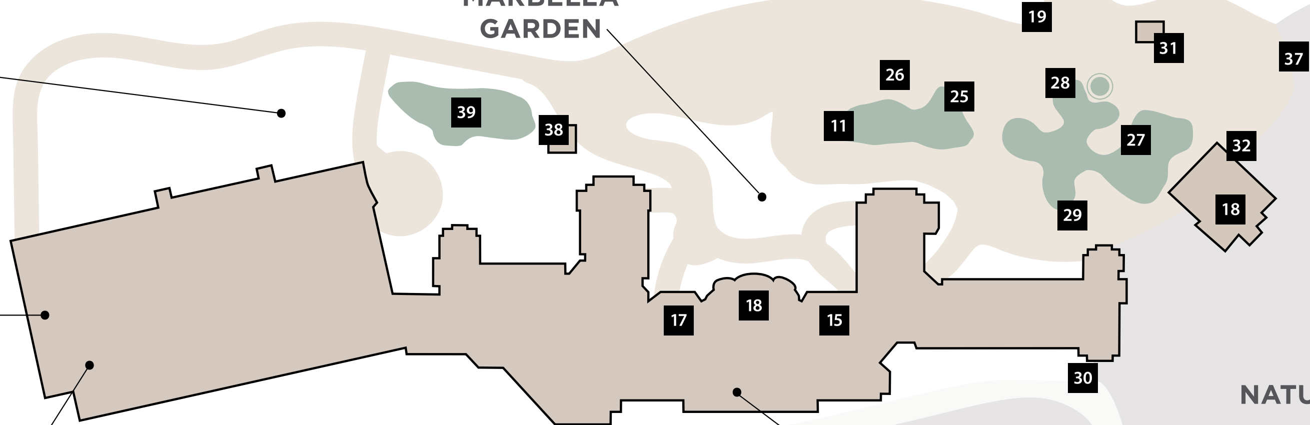

VISTA VERDE GARDEN
MARBELLA GARDEN
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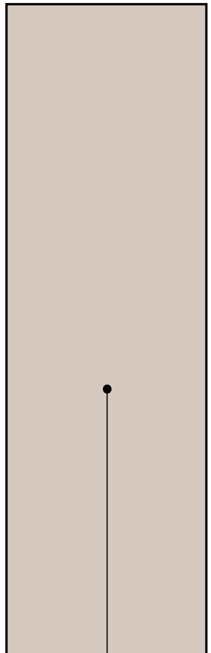
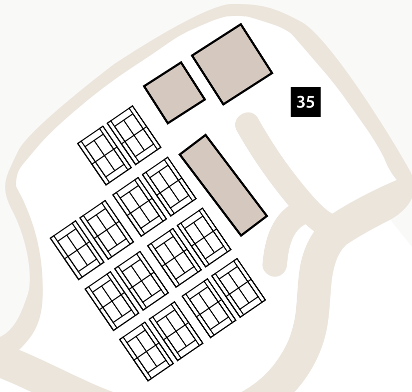
UNDER GROUND SELF-PARKING
CONFERENCE CENTER
NATURE PRESERVE
NATURE PRESERVE
MAIN ENTRY & VALET PARKING



SELF-PARKING



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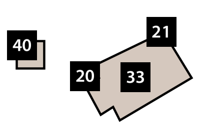

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TOM & GEORGE FAZIO'S OCEAN GOLF COURSE
CLUB HOUSE PARKING
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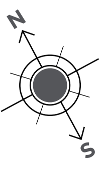
GREG NORMANS RIVER GOLF COURSE
WELCOME GATE



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6. Margaritaville Registration Desk
7. Car Rental
8. Gift Shop & Sundries
9. El Yunque Foyer
10. Business Center
11. Cabana Rentals/ Pool Concierge

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13. Barista Café
14. Boleros
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17. *New Concept Restaurant
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19. Tiki Hut
20. *Iguana's Club
21. *Atrium Bar

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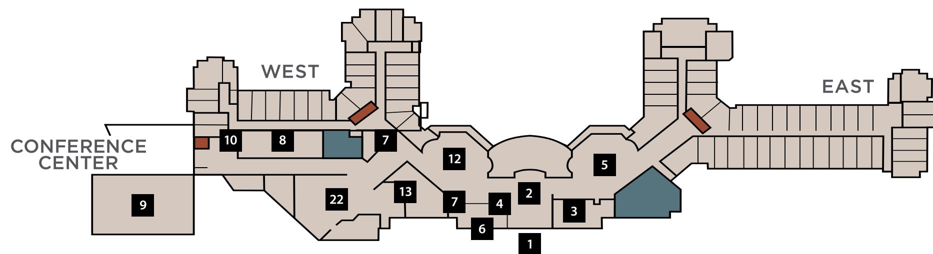
22. Rio Mar Casino
23. Meeting Space
24. Mandara Spa/ Fitness Center
25. Quiet Pool
26. Jacuzzi
27. Activity Pool
28. Jacuzzi
29. Kiddie Pool
30. Iguana's Feeding Area
31. Water Sports Rental
32. Pepsi Pavilion
33. Rio Mar Country Club
34. Driving Range
35. Tennis Center
36. Activity Lounge
37. Beach Deck
38. 5 O'Clock Somewhere Bar & Grill
39. Margaritaville Pool
40. Hole In One

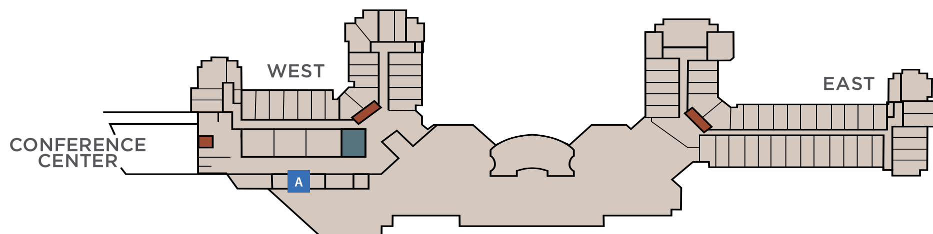
Opening soon

A — Level 1 Meeting Rooms
B — Level 2 Meeting Rooms
C — Conference Center
Restrooms
Elevators

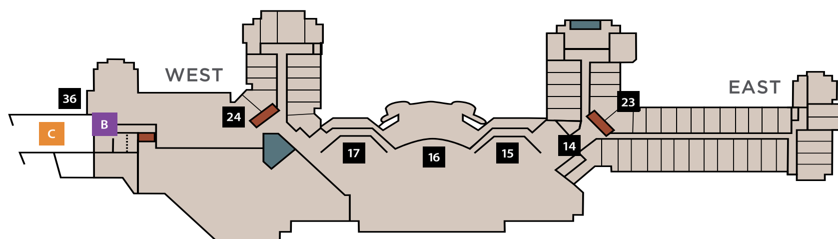
LEVEL 3 - LOBBY LEVEL



LEVEL 2



LEVEL 1 - ACCESS TO THE POOL & BEACH



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