

# The 29<sup>th</sup> International Mammalian Genome Conference

Yokohama, Japan  
November 8-11, 2015



## Program at a Glance

### Saturday, 7<sup>th</sup> November 2015

17.00 - 21.00	Secretariat Meeting	E' Carina
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### Sunday, 8<sup>th</sup> November 2015

09.00 - 16.00	Registration Open at RIKEN	Koryuto Hall, RIKEN
09.30 - 11.45	Bioinformatics workshop	Koryuto Hall, RIKEN
11.30 - 13.55	Optional RIKEN lab tours (11.55 - 12.45 / 12.30 - 13.20 / 13.05 - 13.55)	meet at Koryuto Hall, RIKEN
11.45 - 13.30	Lunch	Cafeteria 2nd floor Koryuto Hall
13.30 - 18.00	Trainee Symposium	Koryuto Hall, RIKEN
19.00 - 21.00	Registration Open at Welcome Reception	Fisherman's Market
19.30 - 21.00	Welcome Reception	Fisherman's Market

### Monday, 9<sup>th</sup> November 2015 (all events at Yokohama Port Opening Memorial Hall)

09.00	Registration Open	
09.00 - 19.30	Poster Space Open (Odd Posters on Display)	Rooms No. 1 & 7
09.15 - 9.30	Opening Greetings	
9.30 - 11.00	Plenary Session: <b>Human Disease Models &amp; Immunology I</b>	
11.00 - 11.30	Break	Rooms No. 4, 6 & 9
11.30 - 13.00	Plenary Session: <b>Neuroscience, Development &amp; Stem Cells</b>	
13.00 - 14.00	Lunch	Rooms No. 4, 6 & 9 and Sei-ren
13.00 - 14.00	Mentor Lunch	E' Carina
14.00 - 15.00	Plenary Session: <b>Genomics and Computational Analysis I</b>	
15.00 - 16.00	IMGS Business Meeting ( <i>All are encouraged to participate</i> )	
16.00 - 18.00	Exhibition & Posters (Odd)	Rooms No. 1 & 7
18.00 - 19.00	Plenary Session: <b>Human Disease Models &amp; Immunology II</b>	
19.30 - 21.30	Systems Genetics Workshop (Optional)	
19.30 - 21.30	Scientific Literature Curation (Optional)	
	Evening free for delegates' own dinner plans	

### Tuesday, 10<sup>th</sup> November 2015

09.00	Registration Open	
09.00 - 19.30	Poster Space Open (Even Posters on Display)	Rooms No. 1 & 7
09.15 - 10.45	Plenary Session: <b>Human Disease Models &amp; Immunology III</b>	
10.45 - 11.35	Mini Session: <b>Is the mouse still needed as a human disease model?</b>	
11.35 - 12.00	Break	Rooms No. 4, 6 & 9

12.00 - 13.00	Plenary Session: <b>Epigenomics and noncoding RNAs I</b>	
13.00 - 14.00	Lunch	Rooms No. 4, 6 & 9 and Sei-ren
13.00 - 14.00	Nomenclature Lunch Meeting	Anteroom
13.00 - 14.00	Luncheon seminar	La bar a vin 52
14.00 - 14.45	<b>Keynote Lecture:</b> Masayo Takahashi	
14.45 - 15.30	Plenary Session: <b>Advances in Genome Editing</b>	
15.30 - 17.30	Exhibition and Posters (Even)/Break	Rooms No. 1 & 7
17.30 - 19.00	Plenary Session: <b>Genomics and Computational Analysis II</b>	
19.30 - 21.30	Gene Enrichment Analysis Workshop (Optional)	
19.30 - 21.30	FANTOM Workshop (Optional)	
	Evening free for delegates' own dinner plans	

### Wednesday, 11<sup>th</sup> November 2015

09.00	Registration Open	
09.15 - 10.30	Plenary Session: <b>Large-scale resources I</b> <b>DMDS Lecture:</b> Janan Eppig	
10.30 - 11.00	Break	Rooms No. 4, 6 & 9
11.00 - 12.00	<b>Verne Chapman Lecture:</b> John Mattick	
12.00 - 13.00	Plenary Session: <b>Epigenomics and noncoding RNAs II</b>	
13.00 - 14.00	Lunch	Rooms No. 6 & 9 and Sei-ren
13.00 - 14.00	Lunchtime Secretariat Meeting	Anteroom
13.00 - 14.00	Luncheon seminar	La bar a vin 52
13.00 - 14.00	Lunchtime Mammalian Genome Editorial Board Meeting	Room. No. 4
14.00 - 14.45	<b>Keynote Lecture:</b> Hideyuki Okano	
14.45 - 15.30	Plenary Session: <b>Large-scale resources II</b>	
15.30 - 16.00	Break	Rooms No. 4, 6 & 9
16.00 - 17.00	Plenary Session: <b>Genomics and Computational Analysis III</b>	
18.00	Bus from YPOMH to Conference Dinner	
19.30	Conference Dinner and Awards Ceremony	The Manyo Club

## Program

### 29<sup>th</sup> International Mammalian Genome Conference (IMGC)

Yokohama, Japan



### Saturday, 7<sup>th</sup> November 2015

17.00 - 21.00	Secretariat Meeting	E' Carina
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### Sunday, 8<sup>th</sup> November 2015

09.00 - 16.00	Registration Open at RIKEN	Koryuto Hall at RIKEN
09.30 - 11.45	Bioinformatics workshop	Koryuto Hall at RIKEN

11.30 - 14.00	Optional RIKEN lab tours (11.55-12.45 / 12.30-13.20 / 13.05-13.55)	meet at Koryuto Hall at RIKEN
11.45 - 13.30	Lunch	Cafeteria 2nd floor Koryuto Hall
13.30 - 18.00	Trainee Symposium	Koryuto Hall at RIKEN
13.30	<a href="#">TS-01</a> : "Development of innovative therapeutic strategy using DNA minor groove binder-drug conjugate in MYCN-driven neuroblastoma", Hiroyuki Yoda	
13.45	<a href="#">TS-02</a> : "Mapping SARS-Coronavirus susceptibility alleles using the Collaborative Cross", Lisa Gralinski	
14.00	<a href="#">TS-03</a> : "Transcriptional control of hibernation: first insights on comparative genomics of dormice", Guzel Gazizova	
14.15	<a href="#">TS-04</a> : "Combination of selective breeding and genome-wide SNP analysis revealed the genetic loci associated with tame behavior in mice ", Yuki Matsumoto	
14.30	<a href="#">TS-05</a> : "Conservation and evolution of splicing patterns during postnatal development of prefrontal cortex in primates", Pavel Mazin	
14.45	<a href="#">TS-06</a> : "Conservative miRNA target analysis: are we limiting our discoveries of neuronal miRNA function?", Belinda J Goldie	
15.00	<a href="#">TS-07</a> : "Dissecting the regulation of olfactory receptor expression in the mouse.", Ximena Ibarra-Soria	
15.15	<a href="#">TS-08</a> : "Horses: an underutilized animal model", Brandon Velie	
15.30 - 16:00	Break	
16.00	<a href="#">TS-09</a> : "Higher expression of <i>Adcyap1</i> gene is associated with altered behavioral and prolonged physiological responses to stress in wild-derived MSM mice", Akira Tanave	
16.15	<a href="#">TS-10</a> : "Analysis of three human genomic loci associated with Tetralogy of Fallot.", Gennadiy Tenin	
16.30	<a href="#">TS-11</a> : "Cognitive Endophenotypes of Modern and Extinct Hominins Associated With <i>NTNG</i> Gene Paralog", Pavel Prosselkov	
16.45	<a href="#">TS-12</a> : "Skin Megagenetics - Novel skin phenotypes revealed by a genome-wide mouse reverse genetic screen", Kifayathullah Liakath-Ali	
17.00	<a href="#">TS-13</a> : " <i>KRAS</i> mutation specific alkylating pyrrole-imidazole polyamide, <i>KR12</i> showed significant anti-tumor efficacy and preferential localization in <i>KRAS</i> mutant xenografts without adverse effects", Takahiro Inoue	
17.15	<a href="#">TS-14</a> : "Development of HTS system to optimize <i>SINEUPs</i> , antisense long-noncoding RNAs that increase translation of target mRNAs", Hazuki Takahashi	
17.30	<a href="#">TS-15</a> : "Cell-cell-communication in cancer", Riti Roy	
17.45	<a href="#">TS-16</a> : " <i>ENU</i> mutagenesis identifies a novel molecular pathomechanism of severe immunodeficiency", Irina Treise	
18.15 -	Bus from RIKEN to Welcome Reception	
19.00 - 21.00	Registration Open at Welcome Reception	Fisherman's Market
19.30 - 21.00	Welcome Reception	Fisherman's Market

### Monday, 9<sup>th</sup> November 2015 (all events at Yokohama Port Opening Memorial Hall)

09.00	Registration Open	
09.00 - 19.30	Poster Space Open (odd numbered posters)	Rooms 1 & 7
09.15 - 9.30	Opening Greetings	
9.30 - 11.00	Plenary Session: <b>Human Disease Models &amp; Immunology I</b>	
9.30	<a href="#">O-01</a> : "Patient Derived Xenografts (PDX) Models of Human Breast Cancer: A Platform for Precision Oncology", Carol J Bult	
9.45	<a href="#">O-02</a> : "Novel <i>ENU</i> -induced ankyrin 1 mutations reveal complex role of erythrocyte cytoskeleton plays during malaria infection", Gaetan Burgio	
10.00	<a href="#">O-03</a> : "Genetic mechanism of aerobic capacity and metabolic disease in Rat model", Yu Wang	
10.15	<a href="#">O-04</a> : "Analysis of murine resistant and susceptible transcriptomes in plague", Jean Jaubert	
10.30	<a href="#">O-05</a> : "Emergence of extreme phenotypes and new disease models in the Collaborative Cross: from bronchiectasis to parkinsonism", Fernando Pardo-Manuel de Villena	
10.45	Invited talk from Trainee Session	
11.00 - 11.30	Break	Rooms 4, 6 & 9
11.30 - 13.00	Plenary Session: <b>Neuroscience, Development &amp; Stem Cells</b>	
11.30	<a href="#">O-06</a> : "Mouse embryonic stem cell lines derived in <i>2i</i> culture conditions demonstrate X Chromosome silencing and extreme male sex bias.", Anne Czechanski	

- 11.45 [O-07](#): "Endogenous L1 Retrotransposition in the Mammalian Early Embryo and Primordial Germline", Sandra R. Richardson
- 12.00 [O-08](#): "The complex transcriptional landscape of the C9orf72 gene locus the most common cause for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)", Patrizia Rizzu
- 12.15 [O-09](#): "Dissection of Genetic Redundancy: A Novel Role for FGF Signaling During Mouse Eye Development", Yasuhide Furuta
- 12.30 [O-10](#): "Molecular biomarkers of neurodegenerative disease in the olfactory epithelium", Gabriela Sanchez-Andrade
- 12.45 Invited talk from Trainee Session
- 13.00 - 14.00 Lunch Rooms 4, 6 & 9 and Sei-ren
- 13.00 - 14.00 Mentor Lunch E' Carina
- 14.00 - 15.00 Plenary Session:  
**Genomics and Computational Analysis I**
- 14.00 [O-11](#): "A tale of two gene sets: low and high variability in single cell RNA-seq data", Anna Mantsoki
- 14.15 [O-12](#): "Single-molecule RNA sequencing in single cells.", Charles Plessy
- 14.30 [O-13](#): "Digital expression profiling of Purkinje neurons and dendrites in subcellular resolution", Anton Kratz
- 14.45 Invited talk from Trainee Session
- 15.00 - 16.00 IMGS Business Meeting (*All are encouraged to participate*)
- 16.00 - 18.00 Exhibition & Posters (Odd) Rooms 1 & 7
- 18.00 - 19.00 Plenary Session:  
**Human Disease Models & Immunology II**
- 18.00 [O-14](#): "Time-dependent host response to influenza A virus infection in Collaborative Cross founder strains and lines", Heike Kollmus
- 18.15 [O-15](#): "A forward-reverse systems genetics approach to understand host-pathogen genetic interactions", Clare Smith
- 18.30 [O-16](#): "Mapping thrombosis modifier genes by bulk exome sequencing mice from a sensitized ENU mutagenesis screen ", Kart Tomberg
- 18.45 Invited talk from Trainee Session
- 19.00 - 19.30 Remove Posters Rooms 1 & 7
- 19.30 - 21.30 Systems Genetics Workshop (Optional)
- 19.30 - 21.30 Scientific Literature Curation (Optional)
- Evening free for delegates' own dinner plans

## Tuesday, 10<sup>th</sup> November 2015

- 09.00 Registration Open
- 09.00 - 19.30 Poster Space Open (even numbered posters) Rooms 1 & 7
- 09.15 - 10.45 Plenary Session:  
**Human Disease Models & Immunology III**
- 9.15 [O-17](#): "A systematic functional screening approach to identify candidate genes obtained through large scale whole genome and exome sequencing of human disease cohorts.", Peter Heutink
- 9.30 [O-18](#): "Natural allelic variation of interleukin 21 receptor modulates ischemic stroke outcomes", Han Kyu Lee
- 9.45 [O-19](#): "Identification of novel susceptibility genes for aggressive prostate cancer using Diversity Outbred mice", Nigel Crawford
- 10.00 [O-20](#): "Strong epistatic control of development of leishmaniasis.", Tatyana Kobets
- 10.15 [O-21](#): "Metabolic regulation by the MECP2 transcriptional repressor complex points to new therapeutic targets in Rett syndrome", Stephanie M Kyle
- 10.30 [O-22](#): "Mouse Models of Human Diaphragmatic Birth Defects: The mesothelium performs a fundamental role in proper formation of the diaphragm", Kate Ackerman
- 10.45 - 11.35 **Mini Session** "Is the mouse still needed as a human disease model?"  
[O-23](#): "Genomic responses in mouse models greatly mimic human inflammatory diseases", Tsuyoshi Miyakawa
- 11.35 - 12.00 Break Rooms 4, 6 & 9

- 12.00 - 13.00 Plenary Session:  
**Epigenomics and noncoding RNAs I**
- 12.00 [O-24](#): "*Bipartite structure of the inactive mouse X Chromosome*", Christine M Disteche
- 12.15 [O-25](#): "*Genetic and dietary effects on gametic selection at fertilization*", Joseph Nadeau
- 12.30 [O-26](#): "*Epigenetic inheritance of diet induced obesity and diabetes via oocyte and sperm*", Johannes Beckers
- 12.45 [O-27](#): "*Exploring regulatory networks through omics data*", S Sethi
- 13.00 - 14.00 Lunch Rooms 4, 6 & 9 and Sei-ren
- 13.00 - 14.00 Nomenclature Lunch Meeting Anteroom
- 13.00 - 14.00 Luncheon seminar La bar a vin 52
- 14.00 - 14.45 **Keynote Lecture:**  
[O-28](#): "*Retinal cell therapy using iPS cells*", Masayo Takahashi
- 14.45 - 15.30 Plenary Session:  
**Advances in Genome Editing**
- 14.45 [O-29](#): "*CRISPR/Cas9 genome editing in rodents: In vivo and in vitro applications*", Marie-Christine Birling
- 15.00 [O-30](#): "*CRISPR/Cas9-mediated plasmid knock-in and replacement of genomic region with single stranded oligonucleotides in rodents*", Kazuto Yoshimi
- 15.15 [O-31](#): "*CRISPR-Driven Replacement of a Mouse Tumor Suppressor with 25-kbp of the Orthologous Human Gene*", David Bergstrom
- 15.30 - 17.30 Exhibition and Posters (Even)/Break Rooms 1 & 7
- 17.30 - 19.00 Plenary Session:  
**Genomics and Computational Analysis II**
- 17.30 [O-32](#): "*The Estimation of Selective Effects Using Large Scale Population Data Identifies Genes Required For Normal Mammalian Development.*", David Beier
- 17.45 [O-33](#): "*The landscape of replication associated mutations in the human and mouse germlines*", Martin Taylor
- 18.00 [O-34](#): "*Paradoxical evolution of a large segmental duplication in mouse*", Andrew P Morgan
- 18.15 [O-35](#): "*The frequent evolutionary birth and death of functional promoters in mouse and human*", Robert Young
- 18.30 [O-36](#): "*Elucidation of the Underlying Mechanism of the Induction of Pluripotency Genes by SAHA-Conjugated Pyrrole-Imidazole Polyamides by Computational Genomic Analysis*", Jason Lin
- 18.45 [O-37](#): "*Capybara genome sequencing offers deeper insights into rodent evolution*", Isaac Adeyemi Babarinde
- 19.00 - 19.30 Remove Posters Rooms 1 & 7
- 19.30 - 21.30 Gene Enrichment Analysis Workshop (Optional)
- 19.30 - 21.30 FANTOM Workshop (Optional)
- Evening free for delegates' own dinner plans

### Wednesday, 11<sup>th</sup> November 2015

- 09.00 Registration Open
- 09.15 - 10.30 Plenary Session:  
**Large-scale resources I**
- 9.15 [O-38](#): **DMSD Lecture** "*The Short Story of a Long Tale*", Janan Eppig
- 9.45 [O-39](#): "*Informatics for the International Mouse Phenotyping Consortium- a Platform for Phenotypic and Translational Discovery*", Terrence F Meehan
- 10.00 [O-40](#): "*Systemic metabolic phenotyping in the German Mouse Clinic in search of new mouse models for metabolic disorders*", Jan Rozman
- 10.15 [O-41](#): "*Genetic architecture of behavior in an advanced intercross line of mice*", Natalia M. Gonzales
- 10.30 - 11.00 Break Rooms 4, 6 & 9
- 11.00 - 12.00 **Verne Chapman Lecture:**  
[O-42](#): "*The hidden layer of regulatory RNA in mammalian genome biology*", John Mattick

12.00 - 13.00	Plenary Session: <b>Epigenomics and noncoding RNAs II</b>	
12.00	<a href="#">O-43</a> : "In vivo profiling of the promoter- and enhancer landscape of inflammatory bowel disease ", Albin Sandelin	
12.15	<a href="#">O-44</a> : "Enhancers lead waves of coordinated transcription in transitioning mammalian cells", Erik Arner	
12.30	<a href="#">O-45</a> : "Network architecture of microRNA regulation in mammalian cells", Michiel De Hoon	
12.45	<a href="#">O-46</a> : "Regulated mobilization of Retrotransposable elements in cell identity, reprogramming and disease", Valerio Orlando	
13.00 - 14.00	Lunch	Rooms 6 & 9 and Sei-ren
13.00 - 14.00	Lunchtime Secretariat Meeting	Anteroom
13.00 - 13.45	Luncheon seminar	La bar a vin 52
13.00 - 14.00	Lunchtime Mammalian Genome Editorial Board Meeting	Room 4
14.00 - 14.45	<b>Keynote Lecture:</b> <a href="#">O-47</a> : "Modeling Psychiatric/Neurological disorders using iPS cell technologies and transgenic non-human primates.", Hideyuki Okano	
14.45 - 15.30	Plenary Session: <b>Large-scale resources II</b>	
14.45	<a href="#">O-48</a> : "GENCODE: revealing transcriptional complexity in Human and Mouse", Mark Thomas	
15.00	<a href="#">O-49</a> : "FANTOM6: Functional elucidation of lncRNA", Jay W Shin	
15.15	<a href="#">O-50</a> : "Multiple mouse reference genomes and strain specific gene annotations", Thomas Keane	
15.30 - 16.00	Break	Rooms 4, 6 & 9
16.00 - 17.00	Plenary Session: <b>Genomics and Computational Analysis III</b>	
16.00	<a href="#">O-51</a> : "A survey of genome rearrangements in human evolution", Martin Frith	
16.15	<a href="#">O-52</a> : "An experimental approach to elucidate enigmatic isochore evolution by using ENU mutagenesis", Satoshi Oota	
16.30	<a href="#">O-53</a> : "Post-translational mechanisms buffer protein abundance against transcriptional variation.", Steven Munger	
16.45	<a href="#">O-54</a> : "Analysis of energy demands during lactation in mouse models", Peter Williamson	
18.00	Bus from YPOMH to Conference Dinner	
19.30 - 22.30	Conference Dinner and Awards Ceremony	The Manyo Club

## Posters

Number	Title
<a href="#">P-001</a>	"Abnormal Innate Immune Responses of ENU-induced Ali18 and Ali14 Mutant Mice Lead to Autoinflammatory Syndrome-like Phenotypes", Koichiro Abe
<a href="#">P-002</a>	"Xist/Tsix expression dynamics during mouse peri-implantation development revealed by whole-mount 3D RNA-FISH", Kuniya Abe
<a href="#">P-003</a>	"Towards the Creation of Reference Transcription Start Site Set (refTSS)", Imad Abugessaisa
<a href="#">P-004</a>	"Somatic variations in healthy skin fibroblasts and their relation to cancer and aging", Alexej Abyzov
<a href="#">P-005</a>	"Elevated canonical Wnt signalling disrupts development of the embryonic midline and can cause Heterotaxy", Ruth Arkell



- 
- [P-006](#) *"Targeted reduction of highly abundant transcript with pseudo-random primers"*, Ophelie Arnaud
- 
- [P-007](#) *"F5-explorer: an interactive webserver for quick gene-oriented browsing of FANTOM5 data "*, Frederik Otzen Bagger
- 
- [P-008](#) *"Multimer formation explains allelic suppression of PRDM9 recombination hotspots "*, Christopher Baker
- 
- [P-009](#) *"Influence of diet on metabolic syndrome in four genetically diverse mouse strains"*, William Barrington
- 
- [P-010](#) *"Characterization and mapping of colimba, a new spontaneous mouse mutation with hair coat abnormalities."*, Fernando Benavides
- 
- [P-011](#) *"Cilia and Ciliopathologies: Impact of new knowledge on our understanding of biology and human disease"*, Judith Blake
- 
- [P-012](#) *"Single-cell transcriptomes of fluorescent, ubiquitination-based cell cycle indicator cells"*, Michael Boettcher
- 
- [P-013](#) *"Analysis of ENU mutant mice indicates the existence of non-exomic thrombosis modifier mutations"*, Marisa A Brake
- 
- [P-014](#) *"Shape-based morphometric analysis of homozygous lethal embryos imaged by micro-CT"*, James M Brown
- 
- [P-015](#) *"The JAX Synteny Browser: A new visualization tool for mouse-human comparative genomics"*, Carol J Bult
- 
- [P-016](#) *"Modeling Binding Affinity of the Multiple Zinc-Finger Protein PRDM9"*, Gregory W Carter
- 
- [P-017](#) *"Mapping Genomic Distributions of Combinatorial Histone Modifications at the Single Molecule Level"*, Jen-Chien Chang
- 
- [P-018](#) *"Zebrafish serves as a disease model system to investigate the embryonic development effects of human parvovirus B19- NS1 and VP1u protein"*, Ju Chang-Chien
- 
- [P-019](#) *"Investigation of defective spermiogenesis in a human XLID mouse model"*, Chun-Yu Chen
- 
- [P-020](#) *"MECOM (EVI1 or PRDM3) maintains neuronal stem cell self-renewal through chromatin control over RBPJ recruitment."*, Elaine KY Chung
- 
- [P-021](#) *"-----The ORFeome Collaboration: A community resource for expression of most human protein-coding genes"*, The ORFeome Collaboration
- 
- [P-022](#) *"KCNQ1 and CFTR act as tumor suppressors in colorectal cancer"*, RT Cormier
- 
- [P-023](#) *"Whole-exome sequencing of ENU-induced mutant mice with BALB/c background"*, TA de Souza
- 
- [P-024](#) *"A selfish genetic element drives recurrent selective sweeps in the house mouse"*, John P Didion
- 
- [P-025](#) *"Implication of truncated CABLES1 in agenesis of the corpus callosum"*, TH Tra Dinh
- 
- [P-026](#) *"Association Analysis of -1997 Polymorphism in Upstream Regulatory Site of COL1A1 with Low Bone Mineral Density: A Study on Iranian Post-menopausal Women"*, Mina Ebrahimi-Rad
- 
- [P-027](#) *"Genetic control of extreme Influenza disease"*, Martin T Ferris
- 
- [P-028](#) *"The environmental toxicant Tetrabromobisphenol-A promotes adipogenesis by downregulating THY1 (CD90) in human mesenchymal stem cells."*, E" Lissa Flores
- 
- [P-029](#) *"Genetics of Meiotic Recombination Rate"*, Jiri Forejt
- 
- [P-030](#) *"Meiotic Arrest, Crossover Patterns, and Hybrid Sterility of [BALB/cJxJF1/Ms] F<sub>1</sub> Male Mice"*, Yasuhiro Fujiwara
- 
- [P-031](#) *"ENU mutation cataloging in the RINEN ENU mutant mouse library using whole exome analysis with Ion Proton sequencer."*, Ryutaro Fukumura
- 
- [P-032](#) *"Correlation of Trp53cor1 and Trp53 expression in the Trp53cor1 gene trap mouse line"*, Riki Furuhashi
-

- [P-033](#) *"Maternal malnutrition alters gene expression, genomic methylation and behavioral phenotypes of progeny"*, Tamio Furuse
- 
- [P-034](#) *"Transcriptional control of hibernation: first insights on comparative genomics of dormice"*, Guzel Gazizova
- 
- [P-035](#) *"Conservative miRNA target analysis: are we limiting our discoveries of neuronal miRNA function?"*, Belinda J Goldie
- 
- [P-036](#) *"Needs of fundamental revision of mouse genome reference sequences."*, Yoichi Gondo
- 
- [P-037](#) *"Mapping SARS-Coronavirus susceptibility alleles using the Collaborative Cross"*, Lisa Gralinski
- 
- [P-038](#) *"SINEUPs: a new class of natural and synthetic antisense long non-coding RNAs that activate translation."*, Stefano Gustincich
- 
- [P-039](#) *"Genome-wide mapping of hyper-acetylated chromatin with a novel antibody in lung cancer cells"*, Lusy Handoko
- 
- [P-040](#) *"Up-regulation of non-coding RNAs in adult and pediatric liver cancers"*, Kosuke Hashimoto
- 
- [P-041](#) *"Dissecting the contribution of genes from the 17q21.31 region in the Koolen-deVries deletion syndrome using the mouse"*, Yann Herault
- 
- [P-042](#) *"Effects of early-life exposure to Western diet on adult activity levels and associated behavioral and physiological traits in mice"*, Layla Hiramatsu
- 
- [P-043](#) *"An atlas of 5' complete transcripts reveals the genomic origins and expression landscape of human long non-coding RNAs"*, Chung-Chau Hon
- 
- [P-044](#) *"CAGE revealed novel biomarker of periodontitis-associated fibroblasts"*, Masafumi Horie
- 
- [P-045](#) *"Evidence of a direct role for endothelial cells in the development of acute leukemias"*, Viive Howell
- 
- [P-046](#) *"Identification of key regulators contributing to the different responses of transforming growth factor beta in A549 cells by single-cell transcriptome"*, Yi Huang
- 
- [P-047](#) *"Dissecting the regulation of olfactory receptor expression in the mouse."*, Ximena Ibarra-Soria
- 
- [P-048](#) *"KRAS mutation specific alkylating pyrrole-imidazole polyamide, KR12 showed significant anti-tumor efficacy and preferential localization in KRAS mutant xenografts without adverse effects"*, Takahiro Inoue
- 
- [P-049](#) *"Optimization of a Mathematical Model for Explanation of Biological Functions Using Monte-Carlo Methods and Parallel Computing"*, Kazuo Ishii
- 
- [P-050](#) *"Molecular and functional characterization of Angelman syndrome patient-derived iPSCs and neurons"*, Mitsuru Ishikawa
- 
- [P-051](#) *"Recurrent Transcriptome Alterations Across Multiple Cancer Types"*, Bogumil Kaczkowski
- 
- [P-052](#) *"Analysis of mutational landscape in protein domains across a broad spectrum of pathological conditions"*, Alexander Kanapin
- 
- [P-053](#) *"Variation in transcriptional response to 1,25-dihydroxyvitamin D3 and bacterial lipopolysaccharide in primary human monocytes"*, Silvia Kariuki
- 
- [P-054](#) *"Versatile instrument for efficient single cell collection and deposition"*, Stanislav Karsten
- 
- [P-055](#) *"The function of non-coding RNA in stem cell maintenance and differentiation"*, Kaori Kashi
- 
- [P-056](#) *"SEC23A functionally compensates for SEC23B deficiency in mice"*, Rami Khoriaty
- 
- [P-057](#) *"Developing mouse models of open angle glaucoma using large scale ENU mutagenesis"*, Stephen C Kneeland
- 
- [P-058](#) *"Establishing new mouse resource, wild-derived heterogeneous stock, which is useful for genetic analysis of tameness and other complex traits"*, Tsuyoshi Koide
- 
- [P-059](#) *"Does inter-subspecific and -specific swapping of Prdm9 ZFA affect recombination and reproduction in mice?"*, Hiromitsu Kono
-



- [P-060](#) *"C1 CAGE: Quantifying coding and non-coding RNAs at single-cell and single molecule level"*, Tsukasa Kouno
- 
- [P-061](#) *"Targeting PIK3CA gene by Pyrrole Imidazole polyamide seco-CBI conjugates in cervical cancer "*, Sakthisri Krishnamurthy
- 
- [P-062](#) *"Role of a novel asRNA in human white adipose differentiation and metabolism"*, Hiroko Kushige
- 
- [P-063](#) *"Transcriptome analysis of controlled and therapy-resistant childhood asthma reveals distinct gene expression profiles"*, Andrew T Kwon
- 
- [P-064](#) *"A mouse model of development syndrome associated with kaptin (Kptn) mutations"*, MO Levitin
- 
- [P-065](#) *"Collateral damage: Identification and characterisation of spontaneous mutations causing deafness from a targeted knockout programme"*, Morag Lewis
- 
- [P-066](#) *"The long non-coding RNA NEAT1 in cell biology, cancer and paraspeckle function"*, Ruohan Li
- 
- [P-067](#) *"Alkaline ceramidase 1 (Acer1) is indispensable for mammalian skin homeostasis and thermoregulation"*, Kifayathullah Liakath-Ali
- 
- [P-068](#) *"Skin Megagenetics - Novel skin phenotypes revealed by a genome-wide mouse reverse genetic screen"*, Kifayathullah Liakath-Ali
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## Abstracts:

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### Student and Post-Doc Presentations

#### TS-01/P-151: Development of innovative therapeutic strategy using DNA minor groove binder-drug conjugate in MYCN-driven neuroblastoma

Hiroyuki Yoda<sup>\*1,2</sup>, Atsushi Takatori<sup>1</sup>, Takahiro Inoue<sup>1,2</sup>, Takayoshi Watanabe<sup>1</sup>, Nobuko Koshikawa<sup>1</sup>, and Hiroki Nagase<sup>1</sup>

<sup>1</sup>Division of Cancer Genetics, Chiba Cancer Center Research Institute

<sup>2</sup>Department of Molecular Biology & Oncology, Graduate School of Medical & Pharmaceutical Sciences, Chiba University

The MYC family gene, *MYCN*, is a basic helix-loop-helix leucine zipper transcription factor, which is associated with diverse cellular processes including growth, proliferation, death, differentiation, metabolism, self-renewal and pluripotency. Amplification of *MYCN* initiates a dynamic process of genomic instability that is linked to tumor initiation. In neuroblastoma, a childhood tumor of the peripheral nervous system, *MYCN* amplification is found in ~25% of neuroblastoma patients and correlates with poor prognosis. Although development of *MYCN* inhibitor has been considered to be attractive for *MYCN*-driven neuroblastoma, no drugs have yet progressed to clinical application. In this study, we have developed a *MYCN* targeting Pyrrole-Imidazole polyamide-alkylating drug conjugate (*MYCN*-Y), designed to bind directly to minor groove of genomic DNA within the coding region of *MYCN*. Treatment of *MYCN*-amplified neuroblastoma cells with *MYCN*-Y significantly suppressed *MYCN* expression at the mRNA and protein levels. Accordingly, *MYCN*-Y caused much higher cell death in *MYCN*-amplified cells than those in *MYCN*-non-amplified cells, leading to cell cycle arrest and apoptosis in response to DNA damage. Intriguingly, FISH analyses demonstrated that *MYCN*-Y impairs the fluorescence intensity of a probe specific for *MYCN* gene loci (2p24.3), suggesting that *MYCN*-Y directly binds to target genomic sites and abrogates DNA binding of *MYCN* probe by alkylating its predetermined target site. Moreover, *MYCN*-Y strikingly inhibited tumor progression in human neuroblastoma xenograft mouse models. Taken together, our findings suggest that

MYCN-Y is promising and an innovative therapeutic drug candidate for aggressive neuroblastomas with *MYCN* amplification.

## TS-02/P-037: Mapping SARS-Coronavirus susceptibility alleles using the Collaborative Cross

Lisa Gralinski<sup>1</sup>, Vineet Menachery<sup>1</sup>, Martin T Ferris<sup>2,3</sup>, Anne Beal<sup>3</sup>, Jessica Plante<sup>1</sup>, Jacob Kocher<sup>1</sup>, Alexandra Schaefer<sup>1</sup>,  
Fernando Pardo-Manuel de Villena<sup>2</sup>, and Ralph Baric<sup>1,3</sup>

<sup>1</sup>Department of Epidemiology, University of North Carolina at Chapel Hill, USA

<sup>2</sup>Department of Genetics, University of North Carolina at Chapel Hill, USA

<sup>3</sup>Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, USA

New systems genetics approaches are needed to rapidly identify host genes and genetic networks that regulate complex disease outcomes. Using genetically diverse animals from incipient lines of the Collaborative Cross mouse panel, we demonstrate a greatly expanded range of phenotypes relative to classical mouse models of SARS-CoV infection including lung pathology, weight loss and viral titer. We selected two surviving strains, CC003/Unc and CC053/Unc, with highly divergent susceptibilities to SARS-CoV for further study. CC003/Unc is highly resistant to SARS-CoV-induced disease as measured by weight loss, despite having a high viral load ( $2.3 \times 10^6$  PFU per lung) at four days post infection (DPI). In contrast, CC053/Unc is highly susceptible to SARS-CoV infection and exhibits extreme weight loss or death despite having a low viral load ( $4.3 \times 10^3$  PFU per lung) at 4 DPI. F1 progeny display an intermediate weight loss phenotype. 300 F2 mice were bred and infected with SARS-CoV; initial phenotyping included measuring daily weight loss, lung titer and lung hemorrhage at D4. Samples have also been collected for future analysis of lung pathology and serum cytokine response. Approximately 7% of mice succumbed to infection and at 4 DPI mice ranged from losing 25% of their body weight to gaining 5% of their starting body weight. Lung titers range from  $1 \times 10^3$  PFU per lung to  $6.7 \times 10^6$  PFU per lung. Hemorrhage was significantly correlated with weight loss ( $p < 0.001$ ) but not with viral load. We are currently genotyping these F2 mice in preparation for QTL mapping to identify host genome regions containing polymorphisms significantly associated with SARS-CoV-induced disease response.



## **TS-03/P-034: Transcriptional control of hibernation: first insights on comparative genomics of dormice**

Guzel Gazizova<sup>\*1</sup>, Kristina Kitaeva<sup>1</sup>, Oksana Tyapkina<sup>2</sup>, Maria Logacheva<sup>1,3</sup>, Olga Bondareva<sup>4</sup>, Leniz Nurullin<sup>2</sup>, Ivan Vikhlyantsev<sup>5</sup>, Akihiko Ishihara<sup>6</sup>, Noriaki Ishioka<sup>7</sup>, and Oleg Gusev<sup>1,8</sup>

<sup>1</sup>Kazan Federal University, Kazan, Russia

<sup>2</sup>KIBB KSC RAS, Kazan, Russia

<sup>3</sup>Lomonosov Moscow State University, Moscow, Russia

<sup>4</sup>Zoological Institute RAS, Saint-Petersburg, Russia

<sup>5</sup>TTEB RAS, Puschino, Russia

<sup>6</sup>Kyoto University, Kyoto, Japan

<sup>7</sup>ISAS, Sagami-hara, Japan

<sup>8</sup>RIKEN, Yokohama, Japan

Hibernation is the hypometabolic state, allowing survival of some species of mammals under severe environmental conditions such as low temperature and food restriction. Remarkably, hibernating animals can stay immobilized for prolonged periods without loss of muscle strength and mass. Genetic control of hypometabolic-associated processes is of key interest, but remains poorly studied, due to the lack of systematic analysis of the activity of genomes of hibernators. In order to establish new genetic model for studying hibernation in mammals, we initiated dormice genome consortium, to sequence and compare genome structure and activity in several species of dormice different in ability to hibernation. At the current step, we analyzed transcriptome profile in two types of muscles (m. soleus, m. EDL) and spinal cord of edible and forest dormice, by using HiSeq Illumina platform and assembled de novo transcriptomes for both species. We determined 49 887 and 52 779 transcripts in edible and forest dormice respectively. We estimated genes, which were upregulated and downregulated during hibernation: most of genes altered by hibernation in edible dormice were found in both muscle types, while in forest dormice the major changes were observed only m. soleus muscle. The key genes, altered in response to hibernation encode ankyrin repeat domain-containing protein, myosin-binding protein and several transcription factors. Finally, using multi-sequence comparison we have analyzed phylogenetic status of dormice in mammals.

The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University and supported by RFBR JSPS\_a №14-04-92116.

**TS-04/P-076: Combination of selective breeding and genome-wide SNP analysis revealed the genetic loci associated with tame behavior in mice**

Yuki Matsumoto<sup>\*1,2,3</sup>, Hirofumi Nakaoka<sup>4</sup>, Jo Nishino<sup>5</sup>, Tatsuhiko Goto<sup>6</sup>, and Tsuyoshi Koide<sup>1,2</sup>

<sup>1</sup>Department of Genetics, SOKENDAI

<sup>2</sup>Mouse Genomics Resource Laboratory, National Institute of Genetics

<sup>3</sup>JSPS Research Fellow

<sup>4</sup>Division of Human Genetics, National Institute of Genetics

<sup>5</sup>Graduate School of Medicine, Nagoya University

<sup>6</sup>College of Agriculture, Ibaraki University

Tame behavior is one of the major elements in domestication and defined as increased interaction of animals with human. Tame behavior can be divided into two component, one is active tame and the other is passive tame. The two components of tame behavior in mice (*Mus musculus*) can be quantified by behavioral assay which our group previously established. To identify genes associated with active tame behavior which is defined as contacting human hand (contacting), we performed selective breeding for contacting using wild-derived heterogeneous stock (WHS), which is a mixed population derived from 8 wild mouse strains originated in various geographic regions. At the 8th generation of the selective breeding, we obtained 16,328 single nucleotide polymorphism (SNP) data of 32 WHS mice in selection population and the 8 founder strains of WHS by using SNP genotyping array. Because the allele frequency associated with contacting should be increased by selective breeding for contacting, and the loci can be identified by using the deviation from hypothetical allele frequency determined by computer simulation based on non-selection model. Therefore we used computer simulation that combined the information of the pedigree of WHS from generation 0 to 8th, polymorphism and position of each SNP, and determined genome-wide thresholds for significant increase of allele frequency. Then we applied the threshold to observed data of selected population. As a result, a candidate SNP on Chromosome 11 exceeded the threshold. Additionally, the result of association analysis using the SNP and contacting in control population was statistically significant. Three candidate genes in Mouse Genomics Informatics database were so far found in the locus which strongly linked with the candidate SNP, suggested that these genes might be associated with increased levels of contacting.

## TS-05/P-077: Conservation and evolution of splicing patterns during postnatal development of prefrontal cortex in primates

Pavel Mazin<sup>\*1,2</sup>, Philipp Khaitovich<sup>1</sup>, and Mikhail Gelfand<sup>2</sup>

<sup>1</sup>Skolkovo Institute of Science and Technology, Moscow, Russia

<sup>2</sup>IITP RAS, Bolshoy Karetny per. 19, Moscow, Russia

Alternative splicing (AS) allows single gene to produce several mRNA through differential exclusion of introns. AS is abundant in higher eucariotes and affects 95% of human genes. Here, we study splicing changes taking place during postnatal prefrontal cortex development based on RNA-Seq data from 74 humans, 44 chimpanzees and 50 macaques. Using these data we created unbiased, not-human-based gene annotation for all three genomes. It allows us to assess for the first time species- and age-related AS variability in primate brains on genome-wide level.

In agreement with previous studies, our results show that inter-species differences are the main source of the splicing variability. We show that 15% (5788) of alternative exons detected in our data are differentially spliced among the three species. In all three evolutionary lineages the main trend was towards increase in proportion of alternative versus constitutive exons. Up to 38% of species-related AS changes can be explained by single nucleotide substitutions in the corresponding splicing sites

6.4% (2477) of alternative exons exhibit significant splicing changes with age. We have found prominent enrichment in functions linked to the neuronal development among corresponding genes. Our analysis shown significantly higher sequence conservation of the age-related exons and their flanking regions than expected by chance. Using motif-enrichment analysis we have shown that several splicing factors such as *MBNL2*, *RBM4*, *YBX1*, *RBFOX2*, *RBM8A* are involved in age-related regulation of cassette exon splicing.

The frequency of the intron retention changes with age in 1029 (14% of tested) genes. There are about two-fold more age-related retained introns in human, than in the other species. Intron retention is more abundant in newborns and drops with age. We shown that the frequency of intron retention does anti-correlate with the gene expression level that points to the possible participation of the intron retention in the age-related gene expression regulation.

## **TS-06/P-035: Conservative miRNA target analysis: are we limiting our discoveries of neuronal miRNA function?**

Belinda J Goldie<sup>\*1,2</sup>, Dan O Wang<sup>1</sup>, and Murray J Cairns<sup>2,3</sup>

<sup>1</sup>Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University

<sup>2</sup>University of Newcastle, Australia

<sup>3</sup>Schizophrenia Research Institute, Sydney Australia

In studies involving miRNA-mediated gene silencing, much emphasis is placed on the reduction of potential "false positive" candidates by limiting target searches to conserved target sites and conserved miRNA. In contrast, the human brain is unique from other species due to higher order functions that are not conserved. Moreover, many studies of learning and memory as well as models of neurological dysfunction are conducted in animals such as rats and mice.

What are we missing out on?

We used genome-wide techniques to investigate the suitability of the human SH-SY5Y neuroblastoma cell line for modelling the expression profile of neurons and showed that use of an appropriate neuronal differentiation paradigm induced expression of mRNAs and miRNAs characteristic of mature neurons. Using this system we studied the subcellular localisation of both species of RNA as well as their responses to neuronal cues for differentiation and depolarisation. Surprisingly, many miRNAs demonstrating strong localisation preference for the neurites and nucleus, as well as those modulated by activity and released in exosomes, were human- or primate- specific. Bioinformatic target functional analyses of these miRNA were severely limited by tools only considering conserved target pairings. In particular, for human-specific miR-638 only 30 conserved targets were predicted. Manual curation of non-conserved targets increased this list to 1290, and revealed putative regulation of synaptic function and gene expression, and indeed over-expression of this miRNA altered the abundance of around 500 loci and severely impacted the neuronal phenotype. Together these findings highlight the importance of an inclusive approach to miRNA target analysis in the neuronal context.

## **TS-07/P-047: Dissecting the regulation of olfactory receptor expression in the mouse.**

Ximena Ibarra-Soria<sup>\*1</sup>, Darren W. Logan<sup>1</sup>, and John C. Marioni<sup>1,2</sup>

<sup>1</sup>Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton-Cambridge, CB10 1SA, United Kingdom

<sup>2</sup>European Bioinformatics Institute (EMBL-EBI), European Molecular Biology Laboratory, Wellcome Trust Genome Campus, Hinxton-Cambridge, CB10 1SD, United Kingdom

Detection of odorants occurs in the main olfactory epithelium (MOE), which contains olfactory sensory neurons (OSNs) that express olfactory receptors (ORs). These bind the odorants and then transmit an electrical signal to the brain. Each OSN expresses only one OR, from a repertoire of over 1,200 genes, and silences all the others. Therefore, the mouse nose has over 1,200 different OSN types, each patterned by a different OR gene. High levels of genomic variation have been reported both in the mouse and human OR repertoire. This is thought to contribute to the unique sense of smell each individual has, but the mechanisms responsible are not known.

We have devised an RNAseq-based approach to quantify the OSN repertoire of three inbred strains of mice (C57BL/6, 129S5 and CAST/EiJ) via their OR gene expression levels. We found that each strain has a unique and reproducible distribution of OSNs in their noses, and that this is directly instructed by genomic variation.

Additionally, OR expression in the MOE is susceptible to olfactory experience. Exposure to an enriched olfactory environment results in the differential expression of dozens of OR genes in a reproducible and specific manner. These changes increase with time and are reversible. These data allows, for the first time, to comprehensively explore and dissect the effects of genetic and environmental variation in the regulation of OR expression and OSN repertoire. Together they generate an olfactory sensory system that is individually unique.

## TS-08/P-144: Horses: an underutilized animal model

Brandon Velie<sup>\*1</sup>, Kim Fegraeus<sup>1</sup>, Merina Shrestha<sup>1</sup>, Anouk Schurink<sup>5</sup>, Liesbeth Francois<sup>4</sup>, Anneleen Stinckens<sup>4</sup>, Sarah Blott<sup>3</sup>, Bart Ducro<sup>5</sup>, Nadine Buys<sup>4</sup>, Sofia Mikko<sup>1</sup>, June Swinburne<sup>2</sup>, Susanne Eriksson<sup>1</sup>, Carl-Johan Rubin<sup>6</sup>, Jennifer Meadows<sup>6</sup>, Leif Andersson<sup>1,6,8</sup>, Lisa Andersson<sup>7</sup>, and Gabriella Lindgren<sup>1</sup>

<sup>1</sup>Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

<sup>2</sup>Animal DNA Diagnostics Ltd, Cambridgeshire, United Kingdom

<sup>3</sup>School of Veterinary Medicine & Science, University of Nottingham, Leicestershire, United Kingdom

<sup>4</sup>Department of Biosystems, Division of Gene Technology, University of Leuven, Leuven, Belgium

<sup>5</sup>Animal Breeding and Genomics Centre, Wageningen University & Research Centre, Wageningen, the Netherlands

<sup>6</sup>Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

<sup>7</sup>Capilet Genetics AB, Oster Skogsta, Vasteras, Sweden

<sup>8</sup>Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, United States of America

Horses provide an opportunity to study unique phenotypes that can lead to fundamental biological insights as well as help to decipher mechanisms underlying biological and disease processes. At present, we have three horse projects with preliminary results that may serve as models for investigating gene functions in mammals. A GWAS of equine insect bite hypersensitivity (IBH), an allergic recurrent seasonal dermatitis classed as a type I and type IV hypersensitive reaction, suggests the importance of two genomic regions on Chromosome 8 (ECA8). An increased knowledge of the genes involved in the manifestation of IBH is expected to not only improve prevention, diagnosis, and treatment of equine IBH, but may also broaden our understanding of the biology underlying type I and type IV hypersensitive reactions across species. Observed in a wide range of species including humans, a second project concerns polydactyly, a genetic defect that presents as an increased number of digits. Preliminary analyses of a family of ponies suggest a recessive mode of inheritance in horses. Through whole-genome re-sequencing of this family (n=5) we aimed to confirm this mode of inheritance and identify the causative locus. Additionally, Delta F<sub>ST</sub> analyses of harness racing breeds have identified specific candidate regions that harbor genes selected for athletic performance. These regions contain genes known to be involved in energy metabolism and cell growth. Genes that regulate energy metabolism and other biological processes that impact racing performance have the potential to improve our understanding of metabolic defects and diseases in horses as well as in other species. At the meeting we will present results from the three aforementioned studies and comment on the fact that in some circumstances the horse may provide unique knowledge of biological pathways that may not otherwise be fully understood.



**TS-09/P-132: Higher expression of *Adcyap1* gene is associated with altered behavioral and prolonged physiological responses to stress in wild-derived MSM mice**

Akira Tanave<sup>\*1,2</sup>, Aki Takahashi<sup>3</sup>, Kenta Sumiyama<sup>4</sup>, and Tsuyoshi Koide<sup>1,5</sup>

<sup>1</sup>Mouse Genomics Resource Laboratory, National Institute of Genetics in Japan

<sup>2</sup>Transdisciplinary Research Integration Center in Japan

<sup>3</sup>University of Tsukuba in Japan

<sup>4</sup>RIKEN Quantitative Biology Center in Japan

<sup>5</sup>Department of Genetics, SOKENDAI

Stress response is behavioral and physiological responses to stressful situation, in which the phenotypic variation is closely linked with the genetic variation among individuals. Wild-derived mouse strain MSM/Ms (MSM) shows higher behavioral responses to stress than laboratory mice in open-field test. Our previous study revealed that the behavioral responses to stress in MSM mice are mapped on Chromosome 17 under the genetic background of laboratory C57BL/6 (B6) mice. Using a series of congenic strains, we successfully mapped a genetic locus into about 2.3 Mb region at the distal end of Chr17, in which only one protein-coding gene *Adcyap1* (*PACAP*) is located. In this study, we examined the association of the *Adcyap1* gene with the behavioral responses to stress. Although non-synonymous mutation was not found in the *Adcyap1* gene between MSM and B6 strains, the *Adcyap1* mRNA and protein levels were significantly increased in hypothalamus of the *Adcyap1* congenic mice. This higher *Adcyap1* expression was considered as one of the reasons the altered behavioral responses to stress because *Adcyap1* is a neuropeptide that regulates stress responses. We next examined physiological response to acute restraint stress. The *Adcyap1* congenic mice showed increased serum corticosterone levels similar with B6 mice immediately after the stress, but the increased serum corticosterone levels were significantly prolonged after 1-2 hour of the stress in the *Adcyap1* congenic mice compared to B6 mice. These results suggests that the altered stress responses may be linked to the altered behavioral responses to stress. In addition, we developed B6 transgenic mice with *Adcyap1* gene derived from MSM strain by using Tol2 transposon system. Now we are conducting behavioral analyses using the transgenic mice. From these results, we will provide some insights into the functional mechanisms of *Adcyap1* that alters behavioral responses to stress through stress response pathway.

## TS-10/P-136: Analysis of three human genomic loci associated with Tetralogy of Fallot

Gennadiy Tenin<sup>\*</sup>, and Bernard Keavney

Institute of Cardiovascular Sciences, University of Manchester, Manchester, UK

Congenital heart diseases (CHD) are defects in the structure of the heart that are present at birth. They are amongst the most common birth defects and found in 9 out of 1000 births. Tetralogy of Fallot (TOF) is one of the most common cyanotic CHD and is considered to be a multigenic condition. TOF involves four anatomical abnormalities of the heart: pulmonary stenosis, overriding aorta, ventricular septal defect and right ventricular hypertrophy. Studies on animal models have linked the development of TOF to defects in cardiac septation and heart valve formation during early cardiogenesis. Our recent GWAS study found significant association of Tetralogy with three genomic loci: a 1.5 Mb haplotype on Chromosome 12q24.12 (contains 24 coding genes), two variants on 10p11.22 (in *NRP1* gene) and two variants on 13q31.3 (in *GPC5* gene). Analysis of eQTL data available for 5 human cell types did not reveal the expression of which genes might be affected by these variants. Analysis of the published data allowed us to eliminate some genes from the candidate list as non cardiac-related. We further narrowed down the candidate genes list upon the analysis of the mRNA expression patterns in the developing mouse heart. Several poorly characterized genes (eg *Gpc5* and *Gpc6*) showed remarkable expression in the endocardial cushions of both outflow tract and atrioventricular canal, which play a critical role in cardiac septation. We then developed the *in vitro* mouse heart culture assay to test for the functional consequences of the gene knock down by siRNA silencing. All this allowed us to identify new cardiac genes which may be involved in the development of the septation defects found in the Tetralogy of Fallot.

## TS-11/P-102: Cognitive Endophenotypes of Modern and Extinct Hominins Associated With *NTNG* Gene Paralogs

Pavel Prosselkov<sup>\*1,2</sup>, Ryota Hashimoto<sup>3,4</sup>, Denis Polygalov<sup>5</sup>, Ohi Kazutaka<sup>3</sup>, Qi Zhang<sup>1</sup>, Thomas J. McHugh<sup>5</sup>, Masatoshi Takeda<sup>3,4</sup>, and Shigeyoshi Itohara<sup>1</sup>

<sup>1</sup>Laboratory of Behavioral Genetics, RIKEN BSI, Wakoshi, 351-0198 Saitama, Japan

<sup>2</sup>Grad School, Dep Vet Medicine, Faculty of Agriculture, Tokyo University, 113-8657 Tokyo

<sup>3</sup>Mol Res Cnt for Child Mental Dev, Uni Grad Sch Child Dev, Osaka Uni, 565-0871 Osaka

<sup>4</sup>Department of Psychiatry, Osaka Uni Grad School of Medicine, 565-0871 Osaka, Japan

<sup>5</sup>Laboratory of Circuit and Behavioral Physiology, RIKEN BSI, Wakoshi, 351-0198 Saitama

A pair of gene paralogs, *NTNG1* and *NTNG2*, sharing identical gene and protein structures and encoding similar proteins, forms a functional complement subfunctionalising (SF) within cognitive domains forming cognitive endophenotypes, as detected by Intellectual Quotient (IQ) test. *NTNG1* SNPs affect either verbal comprehension (VC, rs2218404) or processing speed (PS, rs96501) while *NTNG2* SNPs (rs2149171 and rs2274855) affect working memory (WM) and perceptual organization (PO), and rs1105684 affects verbal and performance IQs (VIQ and PIQ). Regions of interest (ROIs) defined as 21 nu long loci symmetrically embedding the SNPs' areas underwent dramatic evolutionary changes from mice through primates to human gene orthologs. Two G and one T alleles associated with higher IQ scores show an early evolutionary appearance: rs2218404 "G" of *NTNG1* in extinct Mesolithic human Loschbour (8,000 yrs BC); rs2149171 "T" and rs2274855 "G" of *NTNG2* in Neolithic human *◈tzi* (5,300 yrs BC) and chimpanzee (6.3 mln yrs ago), respectively. Two other mutations associated with lower IQ scores are also relatively young: processing speed (PS)-affecting C allele of rs96501 appears ~50,000 yrs BC; WM/PO - affecting A allele of rs2274855 - 8,000 yrs BC. Intensive evolutionary changes resulting in the accelerated evolution of the VC (rs2218404) and WM/PO (rs2274855) ROIs point towards their potential contribution to the human-specific traits. Protein sequence of *NTNG1* is 100% conserved among the archaic and modern extinct hominins while *NTNG2* underwent a recent selection sweep. It encodes a primate-specific S371A/V substitution (emerged ~50,000 yrs ago) and a modern human (5,300 yrs) T346A substitution (located 20 nu downstream of the WM/PO-affecting rs2274855). *NTNG* paralogs SF perturbate "structure drives function" concept and do not obey it neither at the protein nor gene level proposedly forming a so-called "Cognitive Complement (CC)" as a product of gene duplication and subsequent function bifurcation by the gene duplicates.

**TS-12/P-068: Skin Megagenetics - Novel skin phenotypes revealed by a genome-wide mouse reverse genetic screen**

Kifayathullah Liakath-Ali<sup>\*1,2,3</sup>, Valerie Vancollie<sup>4</sup>, Emma Heath<sup>1</sup>, Damian Smedley<sup>4</sup>, Jeanne Estabel<sup>4</sup>, David Sunter<sup>4</sup>, Tia DiTommaso<sup>5</sup>, Jacqueline White<sup>4</sup>, Ramiro Ramirez-Solis<sup>4</sup>, Ian Smyth<sup>5</sup>, Karen Steel<sup>6</sup>, and Fiona Watt<sup>1</sup>

<sup>1</sup>Centre for Stem Cells and Regenerative Medicine, Kings College London, UK

<sup>2</sup>Department of Biochemistry, University of Cambridge, UK

<sup>3</sup>Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, UK

<sup>4</sup>Wellcome Trust Sanger Institute, Cambridge, UK

<sup>5</sup>Department of Anatomy and Developmental Biology, Monash University, Australia

<sup>6</sup>Wolfson Centre for Age-Related Diseases, Kings College London, UK

Permanent stop-and-shop large scale mouse mutant resources provide an excellent platform to decipher tissue phenogenomics. A skin specific high throughput genetic screen on these resources would reveal novel genes involved in skin homeostasis. In this presentation, we show analysis of skin from 538 unselected knockout mouse mutants. We have optimized labeling methods to allow systematic annotation of hair follicle, sebaceous gland and interfollicular epidermal abnormalities using ontology terms from the Mammalian Phenotype Ontology. Of the 50 mutants with an epidermal phenotype, 9 map to human genetic conditions with skin abnormalities. Some mutant genes are expressed in the skin, whereas others are not, indicating systemic effects. In-depth analysis of three mutants, *Krt76*, *Myo5a* (a model of human Griscelli syndrome) and *Mysm1*, provides validation of the screen. High incident of sebaceous gland abnormalities was observed and one mutant showed dietary influence on sebaceous gland architecture. Computational analysis showed strong association of mutants with core signaling pathways such as EGFR, Notch and Wnt. This is the first large scale genome-wide tissue phenotype screen from the International Knockout Mouse Consortium and provides an open access resource for the scientific community.

**TS-13/P-048: KRAS mutation specific alkylating pyrrole-imidazole polyamide, KR12 showed significant anti-tumor efficacy and preferential localization in KRAS mutant xenografts without adverse effects**

Takahiro Inoue<sup>\*1</sup>, Kiriko Hiraoka<sup>1</sup>, Hiroyuki Yoda<sup>1</sup>, Takayoshi Watanabe<sup>1</sup>, Atsushi Takatori<sup>1</sup>, Nobuko Koshikawa<sup>1</sup>, Shigeharu Wakana<sup>4</sup>, Toshikazu Bando<sup>3</sup>, Hiroshi Sugiyama<sup>3</sup>, Toshinori Ozaki<sup>2</sup>, and Hiroki Nagase<sup>1</sup>

<sup>1</sup>Lab. Cancer Genetics, Chiba Cancer Center Res. Inst.

<sup>2</sup>Lab. DNA Damage Signaling, Chiba Cancer Center Res. Inst.

<sup>3</sup>Dep. Chem., Grad. Sch. Sci., Univ. Kyoto.

<sup>4</sup>Japan Mouse Clinic, RIKEN BRC.

Constitutive active mutations of *KRAS* have been shown to be associated with malignant properties of tumors as well as a poor clinical outcome of the patients in colorectal cancers. Unfortunately, yet no effective anti-cancer drug(s) specifically targeting *KRAS* mutations have been developed. Hence, we synthesized an alkylating agent conjugated with the pyrrole-imidazole polyamide (KR12: PIP-CBI), which recognized *KRAS* G12D or G12V mutation at codon12. We have previously found anti-tumor effects of low dose KR12 (0.3mg/kg) by suppressing mutant *KRAS* expression *in vitro* and *in vivo*. However, it still remains elusive the KR12 pharmacokinetics, maximum effective/tolerated doses and safety in animals. To address those, we initially performed Modified SHIRPA (behavioral and functional analysis of mouse phenotype) to test phenotype of ICR mice 3 days and one month after 3 mg/kg KR12 single intravenous administration. Modified SHIRPA screening, and simultaneous hematology, blood chemistry and urine analyses exhibited no KR12 exposure related toxicological changes. Additionally, we examine the distribution of KR12 using FITC labeled PI polyamide. *In vivo* imaging of tumor-bearing mice 72 hours after the administration demonstrated the highest fluorescence intensity in the tumor. Since KR12 showed long lasting accumulation in xenografts we compared the effect of single and multiple administration of KR12 in the homozygous mutant SW480 (*KRAS*<sup>G12V</sup> homozygous mutation) xenograft model. Surprisingly, single treatment of 0.3 mg/kg KR12 induced significant tumor volume reduction as those seen in weekly injections for five to eight weeks. These data suggest that substantial KR12 exposure is well tolerated in mice, and KR12 may accumulate in xenograft tumor tissues and thus induced significant tumor growth inhibition even at low doses.

**TS-14/P-128: Development of HTS system to optimize SINEUPs, antisense long-noncoding RNAs that increase translation of target mRNAs**

Hazuki Takahashi<sup>\*1</sup>, Ana Kozuharova<sup>1</sup>, Harshita Sharma<sup>1</sup>, Diego Cotella<sup>2</sup>, Claudio Santoro<sup>2</sup>, Silvia Zucchelli<sup>3</sup>, Stefano Gustincich<sup>3</sup>, and Piero Carninci<sup>1</sup>

<sup>1</sup>RIKEN Center for Life Science Technologies Japan

<sup>2</sup>Universita del Piemonte Orientale Italy

<sup>3</sup>SISSA Italy

SINEUPs are antisense long non-coding RNAs that are able to specifically stimulate translation of potentially any gene of interest, ultimately leading to increased levels of the protein product. They derive from a new functional class of natural long non-coding RNA transcripts antisense to protein coding genes that we recently discovered. These molecules have been named SINEUPs since their function requires the activity of an embedded inverted SINEB2 sequence to UP-regulate translation in a gene-specific manner. SINEUP activity depends on the combination of two elements present in an RNA molecule: the overlapping region is indicated as the Binding Domain (BD) and is responsible of target specificity; the embedded Inverted SINEB2 element is the Effector Domain (ED) and is required for translation enhancement. In order to screen for SINEUPs with enhanced activity, we established a high throughput screening (HTS) system based on high-resolution automated fluorescent imaging using CeligoS platform. A series of variants were generated in the BD and ED of SINEUP-GFP and activity measured as mean of GFP fluorescence intensity. Our HTS analysis identified a number of short BD variants overlapping around the AUG Kozac region with improved activity. Moreover, with the same platform, we validated ED from additional natural SINEUPs that were found effective in enhancing protein translation. In summary, our automated HTS platform can be used for fast and efficient screening for SINEUP design and optimization and can be applied to virtually any target gene of interest.



## TS-15/P-106: Cell-cell-communication in cancer

Riti Roy<sup>\*1</sup>, Jordan A Ramilowski<sup>2</sup>, and Alistair Forrest<sup>1,2</sup>

<sup>1</sup>Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, the University of Western Australia, PO Box 7214, 6 Verdun Street, Nedlands, Perth, Western Australia 6009, Australia

<sup>2</sup>RIKEN Center for Life Science Technologies (Division of Genomic Technologies), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 Japan.

To coordinate multicellular processes, cells need to communicate. One way they communicate is through receptor and ligand interactions. By studying the peptide ligands and plasma-membrane receptors expressed on different primary cell types we have generated the world's first draft map of the cell-cell communication network CCCN (*Ramilowski et al. 2015*). Here we explore what happens to cell-cell communication in tumors (in particular cancer-cancer and cancer-normal signalling). Examining the expression profiles of receptors and ligands in cell lines profiled by the FANTOM5 project and tumors profiled by the TCGA we report on changes to the ligand and receptor repertoires expressed in cancer and relate this to mutation profiles. We also examine changes in cell-cell communication in cancer, with a systematic reevaluation of cancer-autocrine, cancer-stroma, cancer-vascular and cancer-immune signalling. This area of research is important as it gives new insights into the complex network of cell-cell communication in tumors and has the potential to identify new therapeutic targets.

## TS-16/P-138: ENU mutagenesis identifies a novel molecular pathomechanism of severe immunodeficiency

Irina Treise<sup>\*1,2</sup>, Eva M. Huber<sup>3</sup>, Tanja Klein-Rodewald<sup>1,7</sup>, Wolfgang Heinemeyer<sup>3</sup>, Thure Adler<sup>1,2</sup>, Birgit Rathkolb<sup>1,4</sup>, Christian Andres<sup>8</sup>, Thomas Wieland<sup>6</sup>, Tim M. Strom<sup>6</sup>, Kathy D. McCoy<sup>5</sup>, Andrew J. Macpherson<sup>5</sup>, Eckhard Wolf<sup>4</sup>, Markus Ollert<sup>8</sup>, Frauke Neff<sup>7</sup>, Valerie Gailus-Durner<sup>1</sup>, Helmut Fuchs<sup>1</sup>, Martin Hrabe de Angelis<sup>1</sup>, Michael Groll<sup>3</sup>, and Dirk H. Busch<sup>2</sup>

<sup>1</sup>German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum Muenchen, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

<sup>2</sup>Institute for Medical Microbiology, Immunology and Hygiene, Technische Universitaet Muenchen, 81675 Munich, Germany

<sup>3</sup>Center for Integrated Protein Science at the Department Chemistry, Chair of Biochemistry, Technische Universitaet Muenchen, Lichtenbergstr. 4, 85747 Garching, Germany

<sup>4</sup>Institute for Molecular Animal Breeding and Biotechnology, Gene Center of the Ludwig-Maximilians-Universitaet Muenchen, Feodor-Lynen Str. 25, 81377 Munich, Germany

<sup>5</sup>University Clinic of Visceral Surgery and Medicine, Departement Klinische Forschung, University of Bern, Murtenstrasse 35, CH-3010 Bern, Switzerland

<sup>6</sup>Institute of Human Genetics, Helmholtz Zentrum Muenchen, Ingolsaedter Landstr. 1, 85764 Neuherberg, Germany

<sup>7</sup>Institute of Pathology, Helmholtz Zentrum Muenchen, Ingolsaedter Landstrasse 1, 85764 Neuherberg, Germany

<sup>8</sup>Department of Dermatology and Allergy Biederstein, Technische Universitaet Muenchen, Biedersteiner Str. 29, 80802 Munich, Germany

As part of the large-scale Munich ENU (*N*-ethyl-*N*-nitrosourea) mouse mutagenesis approach, the Immunology Screen of the German Mouse Clinic performed standardized immuno-phenotyping of mouse mutants with a particular focus on identifying mutants with clinical phenotypes. Thereby, we established and characterized a novel mouse mutant - TUB006 - with defects in both innate and adaptive immunity. Heterozygous TUB006 animals show significantly reduced T cell frequencies, and fail to induce a sufficient T cell response to *Listeria monocytogenes*, resulting in lethality upon low-dose infection. Homozygous TUB006 mutants display an early lethal phenotype presenting with severe combined immunodeficiency (SCID) lacking all three major types of lymphocytes: T cells, B cells and NK cells. Furthermore, homozygous mutants develop sterile autoinflammation characterized by granulocytosis and infiltration of neutrophil granulocytes into various organs. Whole exome sequencing unraveled the underlying point mutation in *Psmb10*, encoding a catalytic subunit of the immunoproteasome and thymoproteasome. Yeast mutagenesis and crystallographic data suggest that the severe TUB006-phenotype is caused by structural changes that prevent the biogenesis of functional immuno- and thymoproteasomes. The severe immunodeficiency in TUB006 mice is surprising, since *Psmb10*-knockout mice are healthy, displaying only a subtly altered T cell repertoire. Our data once more show the power of ENU to identify unknown gene functions by creating not only loss-of-function mutants but also gain-of-function, hypo-/hypermorphs or dominant-negative mutations. The identification of causative mutations in mice with clinical phenotypes can directly lead to the discovery of human disease genes. Given the high structural and functional conservation of proteasome subunits between mammals, our data point out the high potential of *Psmb10* mutations to be of clinical relevance in humans with immunological defects of unknown etiology.

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## Panel Discussions

### Panel: Is the mouse still needed as a human disease model?

Shigeharu Wakana<sup>\*</sup>

RIKEN

The use of mice as human disease models is an interesting topic. Some scientists believe that the mouse is not appropriate as a model for innovative drug development and basic medical research. We think that the advantage of using mouse models for research on human diseases needs to be emphasized. Recently the article entitled "Inflammation debate reignites" is enclosed in the journal *Science*. The author claimed that the mouse was a poor model for inflammation research in the field of human medicine (Junhee Seok et al., *PNAS*, 2015). On the other hand, some Japanese scientists have reanalyzed data from the same paper using different assumptions and statistical techniques and concluded that the conclusions drawn were incorrect. Finally, they reported that the mouse models of inflammation do mimic the human conditions (Takeo et al., *PNAS*, 2015). These debates were included in the newspapers *EurekAlert*, *SCIENCE CODEX*, *Medical Xpress*, *GEN News*, and *Science*.

In this meeting, we will invite the Japanese scientist Dr. Miyawaka and ask him to describe his analysis of the inflammation-related data of the mouse and his opinion on the advantage of using the mouse as a human disease model. We also invite Drs. Martin Hrab<sup>◆</sup> de Angelis and Gao Xiang to give commentary on his talk. Finally, we hope to promote a debate in the audience on this topic.

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## Oral Presentations

### **O-01: Patient Derived Xenografts (PDX) Models of Human Breast Cancer: A Platform for Precision Oncology**

Carol J Bult<sup>\*</sup>, James Keck, Susan Airhart, and Joel H Graber

The Jackson Laboratory, Bar Harbor, ME, USA

Precision oncology promises to deliver better outcomes for cancer patients by treating individuals with drugs that are selected based on the genetic and/or genomic profile of their tumor. This genome-guided therapy approach is proving effective in many cancers including, non small cell lung cancer, melanoma, lymphoma, leukemia and breast cancer, each of which has molecular subtypes that respond to targeted treatment. Triple-negative breast cancer (TNBC) patients have not benefited from tailored therapies because the tumors of these patients do not express the markers that make a targeted approach possible. Although TNBC patients often respond well to first line standard chemotherapy in a neoadjuvant setting, the rate of relapse is >50%. No standard treatment options exist for these patients for recurrent disease. Clinically relevant mouse models have the potential to accelerate understanding of the genetic and genomic basis of TNBC and to advance new treatment paradigms.

We have developed a resource of PDX models by implanting human tumors into immunodeficient NOD.Cg-*Prkdc<sup>scid</sup>* *Il2rg<sup>tm1Wjl</sup>/SzJ* (aka, NSG) mice. PDX models provide a powerful *in vivo* platform for testing standard of care and novel therapeutic options in TNBC patients. Human tumors that successfully engrafted were characterized for somatic mutations, copy number variants, and gene expression. Tumor bearing mice for the TNBC PDX models were treated with docetaxel, cisplatin, cyclophosphamide and doxorubicin. Preliminary computational analyses of tumor responses to these treatment regimes using a machine learning approach revealed systematic differences that can be correlated with genome properties of the tumors.

## O-02: Novel ENU-induced ankyrin 1 mutations reveal complex role of erythrocyte cytoskeleton plays during malaria infection

Hong Ming Huang<sup>1</sup>, Andreas Greth<sup>1</sup>, Patrick Lelliott<sup>1</sup>, Shelley Lampkin<sup>1</sup>, Denis Bauer<sup>2,3</sup>, Brendan McMorran<sup>1</sup>, Simon Foote<sup>1</sup>, and Gaetan Burgio<sup>\*1</sup>

<sup>1</sup>The John Curtin School of Medical Research, The Australian National University, ACT 2601, Australia

<sup>2</sup>Preventive Health Flagship, CSIRO, NSW 2113, Australia

<sup>3</sup>Computational Informatics, CSIRO, NSW 2113, Australia.

Malarial parasite resistance to all known antimalarial drugs is now the norm. Parasites develop resistance through modification of both target and intra-parasitic drug concentrations. We have developed a strategy to develop new therapies that will bypass both these mechanisms of resistance. ENU mutagenesis is used to introduce mutations into the germline of mice that are otherwise susceptible to murine malaria. Mice carrying protective mutations will survive a malarial challenge whereas all other mice will succumb. The genes harbouring the mutations are identified and assessed as potential antimalarial drug targets. At present we have identified 40 mutations conferring resistance to malarial infection and have 100 resistant lines. Here, we propose novel mechanisms of host resistance to malaria infection. Through our dominant large scale ENU screen for abnormal red blood cell count, 3 novel mutant alleles were identified in ankyrin 1, erythroid (*Ank1*). We hypothesized that depending on the location of mutation, varying degree of malaria susceptibility and perhaps different mechanisms of resistance could be observed. All of the mutants exhibit an abnormal red blood cell (RBC) count. However, some strains exhibited a shorter RBC half-life and deformed morphology responsible for hereditary spherocytosis disease. Most *Ank1* mutant mice were resistant to the malaria parasite *P. chabaudi*. The RBCs from *Ank1* mutant mice were resistant to parasite invasion, some might impair parasite growth and some were prone to clearance. Different *Ank1* mutations cause changes on erythrocyte properties and malaria resistance with different severity. Truncation-causing mutations seem to affect parasite maturation while substitution causes increased clearance. This study provides the first evidence that multiple mutations in *Ank1* can lead to different mechanisms of susceptibility to malaria in mice, and shows that *Ank1* is essential for supporting the growth and the replication of *Plasmodium* spp within RBCs. Finally *Ank1* is a promising targeted for anti-malarial therapies.

### **O-03: Genetic mechanism of aerobic capacity and metabolic disease in Rat model**

Yu-yu Ren<sup>1</sup>, Yu Wang<sup>\*1</sup>, Lauren G Koch<sup>2</sup>, Steven L Britton<sup>2</sup>, Nathan R Qi<sup>3</sup>, Mary K. Treutelaar<sup>3</sup>, Charles F Burant<sup>3</sup>, and Jun Z Li<sup>1</sup>

<sup>1</sup>Department of Human Genetics

<sup>2</sup>Department of Anesthesiology

<sup>3</sup>Department of Internal Medicine, University of Michigan, Ann Arbor, MI, United States

Aerobic capacity refers to the max amount ability of oxygen consuming during exercise. It is a function of overall performance of cardiorespiratory system, and has strong association of risks of obesity, hypertension, and type-2 diabetes. Better understanding of the mechanism of aerobic capacity could promote the therapy of those disease in human. Existing studies on human populations have been largely limited to the complex background and finite sample size, yet further functional validation is insufficient to success in animal model. Indeed, animal model have a quite different genetic background. Here we adopted a rat model system, comprising with two lines with well established phenotype on running ability, to have a better understanding of the molecular mechanism of aerobic capacity and genetic dynamics during selection. The two lines, HCR/Mco (high-capability runners) and LCR/Mco (low-capability runners), shared a same founding group and inbred with rotational matings. Then, they were selected toward divergent running ability for more than 30 generations, and were both genetically and phenotypically heterogenous. Finally, the HCR/Mco lines displayed a significant improvement of aerobic capacity (the maximum distance of HCR/Mco is 9 fold of that of LCR/Mco). Meanwhile, dramatic phenotypic differences were observed on body weight, blood pressure, and life span between HCR/Mco and LCR/Mco lines. To decipher the genetic mechanism of aerobic capacity, we applied comprehensive tools, including custom SNP array and large scale sequencing to genotype F1 and F2. We genotyped 616 F2 and 56 F1 with ~800K custom Affymetrix arrays and archived an average read depth of ~37X on 40 samples with NGS sequencing. Combined with sequencing and microarray genotype data, we identified four regions highly associated ( LOD >= 5 ) with aerobic capacity. Furthermore, the dynamics of haplotype and recombination was described based on pedigree data. Our result provided a better understanding of metabolic mechanism and genetic background of HCR/Mco-LCR/Mco rat lines, which can serve as an excellent model for therapy of metabolic disease.

#### O-04: Analysis of murine resistant and susceptible transcriptomes in plague

Manal Khalife<sup>1,2,4</sup>, Odile Sismeiro<sup>3</sup>, Jean-Yves Coppee<sup>3</sup>, Marie-Agnes Dillies<sup>3</sup>, Bernd Jagla<sup>3</sup>, Elisabeth Carniel<sup>4</sup>, Christian Demeure<sup>4</sup>, Steven Munger<sup>5</sup>, Jean-Jacques Panthier<sup>1,2</sup>, and Jean Jaubert<sup>\*1,2</sup>

<sup>1</sup>Institut Pasteur, Mouse Functional Genetics Unit, F-75015 Paris, France

<sup>2</sup>CNRS URA 2578, F-75015 Paris, France

<sup>3</sup>Institut Pasteur, Transcriptome and Epigenome, F-75015 Paris, France

<sup>4</sup>Institut Pasteur, Yersinia Unit, F-75015 Paris, France

<sup>5</sup>The Jackson Laboratory, Bar Harbor, Maine 04609, US

Plague is caused by the Gram-negative bacterium *Yersinia pestis*. Laboratory mice such as C57BL/6J (B6) are susceptible to plague. We have described that wild-derived *Mus spretus* SEG/Pas (SEG) mice are exceptionally resistant (90%) to the virulent CO92 wild-type strain of *Y. pestis* in an experimental model of bubonic plague. We identified three genomic regions controlling this resistance, and characterized immunological, histological and cellular differences between SEG strain and B6 reference susceptible strain (Blanchet et al. 2011). To gain further insight in the understanding of this exceptional resistant phenotype, we have decided to explore by RNA-seq the modifications of the mouse transcriptome upon infection in both resistant (SEG) and susceptible (B6) context.

Spleen of control non-infected and infected mice were collected at day 3 post-infection. Day 3 was chosen as time-point for tissue collection as at day 4 there are already clear-cut histological differences in the spleen between the two strains and a marked expansion of a specific macrophage sub population (F4/80+ CD11b-) in SEG mice (Demeure et al. 2012). Important variations in spleen CFU counts were observed between same strain animals collected at day 3, in correlation with a previous study that suggested a high variability in the kinetics of infection (Nham et al. 2012). Animals were therefore divided in 2 groups per strain, based on their spleen CFU counts (Low with CFU count  $\sim 10^3$  CFU/g and High with CFU count  $> 10^5$  CFU/g) and compared with non-infected animals. In order to avoid any species-specific transcripts misalignments, B6 and SEG RNAseq reads were aligned respectively on mm9 reference genome or a *Mus spretus* pseudogenome constructed with Seqnature software (Munger et al. 2014). Resistant SEG contrary to B6 susceptible mice display an important activation of genes implicated in innate immune response in Low group whereas both strains display such activation in High group.

## **O-05: Emergence of extreme phenotypes and new disease models in the Collaborative Cross: from bronchiectasis to parkinsonism**

Fernando Pardo-Manuel de Villena \*

Department of Genetics, University of North Carolina, Chapel Hill, NC, USA

When the Collaborative Cross was conceived over a decade ago it was proposed that one of the key features of this genetic reference population would be the presence of novel pairwise combinations of alleles at many loci and that these unique combinations would lead to the emergence of both novel phenotypes and new models of human disease. That prediction was initially confirmed in 2014 with the report of spontaneous colitis in one CC inbred strain (CC011/Unc) and the mapping of multiple interacting QTL. Since then, there has been an explosion of such findings for a wide spectrum of phenotypes ranging from susceptibility to infectious agents to basic physiology. In addition, it has also become apparent that the vast majority of the CC lines that were initially started were incompatible with life or fertility and that completed CC inbred strains are generally "unfit". Based on findings in both extinct and extant CC lines we propose that epistasis between alleles from different subspecies underlies the prevalence and severity of disease phenotypes in the CC. We will discuss this hypothesis in the light of our characterization of two new models of human disease: drug induced parkinsonism and severe bronchiectasis.



## **O-06: Mouse embryonic stem cell lines derived in 2i culture conditions demonstrate X Chromosome silencing and extreme male sex bias.**

Anne Czechanski<sup>\*</sup>, Candice Byers, Whitney Martin, and Laura G. Reinholdt

The Jackson Laboratory, Bar Harbor, ME 04609

The establishment of pluripotent mouse embryonic stem cells (mESC) from recalcitrant strains is now possible through derivation in the presence of the inhibitor cocktail commonly known as 2i. Together, CHIR99021, a glycogen synthase kinase-3B inhibitor, and PDO325901, a MEK inhibitor, overcome recalcitrance by shifting metastable mESCs from the primed to the pluripotent ground state. We have derived over 150 novel mESC lines using 2i culture conditions and observed a striking male sex bias (96%), regardless of strain background. These results are unexpected given the reported female bias in human ES cell derivation and more recent data suggesting that two X Chromosomes promote pluripotency through the same mechanism as 2i in mESCs. To understand the timing and etiology of male sex bias in 2i derivation conditions, we collected 500 blastocysts from Tg(CAG-EGFP)D4Nagy/J sires and sorted by sex on the basis of EGFP expression. 250 embryos per sex were plated in either 2i+LIF or serum+LIF and cultured under our standard protocols. Prior to disaggregation, no differences in the progression of male and female outgrowths were observed. However, by day 12 in culture, we observed attrition of 80% of female 2i lines, compared to 34% of male 2i lines. In contrast, no sex bias was observed in serum+LIF conditions. Coincident with attrition, emergent female 2i mESC lines exhibited slow growth and poor colony morphology. Quantitative analysis of EGFP expression confirmed loss of EGFP expression consistent with XCI in female lines, a prerequisite for exit from the pluripotent ground state. We hypothesize that the combination of X Chromosome dosage and 2i culture conditions prevent the establishment of a stable ground state in emergent mESC lines eventually leading to XCI in the majority of female mESC lines. We are currently exploring these events through transcriptional profiling of transitory female cell lines.

## O-07: Endogenous L1 Retrotransposition in the Mammalian Early Embryo and Primordial Germline

Sandra R. Richardson<sup>\*1</sup>, Daniel J. Gerhardt<sup>1</sup>, Francisco J. Sanchez-Luque<sup>1,2</sup>, Patricia Gerdes<sup>1</sup>, J. Samuel Jesuadian<sup>1</sup>, Marie-Jeanne H.C. Kempen<sup>1</sup>, Gabriela-Oana Bodea<sup>1</sup>, and Geoffrey J. Faulkner<sup>1,3</sup>

<sup>1</sup>Mater Research Institute-University of Queensland, TRI Building, Woolloongabba QLD 4102, Australia

<sup>2</sup>Pfizer-Universidad de Granada-Junta de Andalucía Centre for Genomics and Oncological Research (Genyo). P.T. Ciencias de la Salud. Avda. de la Ilustración 114, 18016 Granada, Spain

<sup>3</sup>Queensland Brain Institute, University of Queensland, Brisbane QLD 4072, Australia

Long Interspersed Element 1 (LINE-1 or L1) is a mobile genetic element or "jumping gene" presently active in mammals. The average human genome harbours ~80-100 active L1 copies, while mice contain ~3,000 per individual. By virtue of their replicative mobilisation strategy—a process termed retrotransposition—L1 sequences have accumulated to occupy approximately 17% and 18% of human and mouse genomic DNA, respectively. L1 insertions within and proximal to genes can impact gene expression in a variety of ways, and retrotransposition events are frequently associated with duplication, deletion, and rearrangement of genomic sequences. Thus, L1 is a potent endogenous mutagen and an arbiter of genome diversification. In order to exert an ongoing impact on the genomic landscape of a species, new L1 insertions must occur in cells that will contribute their genetic material to subsequent generations—i.e., within the germ lineage or in the pluripotent cells of the early embryo, prior to germline specification. Previous studies have suggested that the early embryo is a prominent milieu for L1 retrotransposition; however, systematic study of the frequency and developmental timing of heritable L1 retrotransposition events has been technically challenging. Here, we have adapted retrotransposon capture sequencing (RC-seq) to detect retrotransposon insertions in mouse genomes, and applied this technique to identify *de novo* heritable L1 insertions in multi-generation pedigrees of C57BL/6 mice. We find that such insertions arise at a rate of approximately 1 in 8 mice. Using a PCR genotyping strategy to deduce the developmental timing of these events, we find evidence consistent with L1 retrotransposition in the early embryo resulting in somatic and germline genetic mosaicism, as well as in early primordial germ cells (PGCs), giving rise to germline-restricted genetic mosaicism. Our findings shed new light on the frequency and developmental origins of the ongoing retrotransposition events continuously shaping mammalian genomes.

## O-08: The complex transcriptional landscape of the *C9orf72* gene locus the most common cause for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)

Patrizia Rizzu<sup>\*1</sup>, Cornelis Blauwendraat<sup>1</sup>, Sasja Heetveld<sup>1</sup>, Emily M Lynes<sup>1</sup>, Melissa Castillo-Lizardo<sup>1</sup>, Ashutosh Dhingra<sup>1</sup>, Elwira Pyz<sup>1</sup>, Matthias Synofzik<sup>1,2</sup>, Margherita Francescatti<sup>1</sup>, and Peter Heutink<sup>1</sup>

<sup>1</sup>German Center for Neurodegenerative Diseases (DZNE), Tuebingen, Germany

<sup>2</sup>Department of Neurodegenerative Diseases, Hertie Institute for Clinical Brain Research, University of Tuebingen, Germany

The non-coding hexanucleotide repeat expansion (HRE) in the *C9orf72* gene is a major cause for ALS/FTD. Several studies suggest the disease might occur through different, not necessarily exclusive mechanisms: a loss of function of *C9orf72* due to haploinsufficiency or a gain of function mechanism mediated by aggregates of bidirectionally transcribed HRE-RNA and unconventionally non-ATG-translated di-peptide proteins.

The loss of function is supported by decreased *C9orf72* mRNA expression in patients but emerging evidence suggests that the gain of function is sufficient for neurodegeneration. Haploinsufficiency, though not the major culprit, could be still detrimental to cells leading to defects in endosomal and autophagic processes. In this context it becomes important to fully understand how *C9orf72* expression is regulated. We therefore first surveyed the *C9orf72* locus in the context of the FANTOM5 project.

*C9orf72* was very highly expressed in myeloblasts, as compared to brain and other tissues. The expression profile of *C9orf72* transcription start sites (TSSs) showed a complex architecture with the two TSSs for the annotated *C9orf72* transcripts composed by tag clusters differentially expressed, between myeloblasts and brain tissues hinting to a *C9orf72* transcript specific cell and/or tissue function which is corroborated by our weighted gene co-expression analysis on myeloblasts and brain data.

We detected new non-annotated TSSs in the sense and antisense strand at the *C9orf72* locus, 5' end of the annotated *C9orf72* transcripts and we confirmed their expression in brain tissues and myeloblasts cells from patients and control donors. The TSSs antisense to *C9orf72* are particularly interesting as they suggest the presence of natural head-to-head antisense transcripts to *C9orf72* mRNA that can provide an additional level of regulation of gene expression. Our qPCR experiments indeed indicate that *C9orf72* and the antisense transcripts negatively correlate in C9 and interestingly in other neurodegenerative diseases patients moreover suggesting *C9orf72* plays a common role in neurodegeneration.

## O-09: Dissection of Genetic Redundancy: A Novel Role for FGF Signaling During Mouse Eye Development

Takaya Abe, Yui Yamashita, Yoshiko Mukumoto, Atsumi Denda, Mari Kaneko, Megumi Watase, Hiroshi Kiyonari, and Yasuhide Furuta\*

Laboratories of Animal Resource Development and Genetic Engineering (LARGE), RIKEN Center for Life Science Technologies (RIKEN CLST), Kobe, Japan

The fibroblast growth factor (FGF) signaling system comprises a battery of ligands and receptors that are promiscuous in their binding partners. Due to apparent functional redundancy, the collective function of endogenous FGF family ligands in embryonic development has not been fully understood genetically. To examine the role of FGF signaling in the developing eye, we are generating mice lacking the function of multiple *Fgf* gene family members, *Fgf3*, *Fgf9*, and *Fgf15*, expressed in the developing retina. Homozygous mutants lacking either of these genes individually exhibit subtle or no morphological defects during early eye development. While combining multiple mutations is required to better understand the function of these genes, generation of *Fgf3;Fgf9;Fgf15* compound mutants by conventional genetic crosses have been hampered due to suboptimal fertility of *Fgf9* mutants and a close genetic linkage between *Fgf3* and *Fgf15* loci on the same chromosome. To circumvent these problems, we have employed CRISPR/Cas9-mediated multi-gene targeting to generate compound mutant embryonic stem (ES) cells. Mutant ES cells were used to generate completely ES-derived Fo chimeras for direct phenotypic characterization. Preliminary phenotypic analyses have revealed that embryos lacking the functions of both *Fgf9* and *Fgf15* show misrouting of retinal ganglion cell (RGC) axons soon after the initiation of RGC differentiation. Subsequently, RGC axons fail to target the central retina, resulting in the absence of the optic nerve. These results suggest a previously unrecognized role of FGF signaling during retinal development, apparently controlling RGC axon behaviors to properly target the central retina for the formation of the optic nerve. Further phenotypic analyses of mutants with various *Fgf3;Fgf9;Fgf15* compound genotype combinations will also be reported. These approaches have provided us with a stable source of compound mutants, and thus will allow for efficient genetic analyses of lethal multi-gene mutations.

## O-10: Molecular biomarkers of neurodegenerative disease in the olfactory epithelium

Gabriela Sanchez-Andrade<sup>\*1</sup>, Rishika Kundra<sup>2</sup>, Sujeong Yang<sup>3</sup>, Michel Goedert<sup>4</sup>, Maria Grazia Spillantini<sup>3</sup>, Michele Vendruscolo<sup>2</sup>, and Darren W. Logan<sup>1</sup>

<sup>1</sup>Wellcome Trust Sanger Institute

<sup>2</sup>Department of Chemistry, University of Cambridge

<sup>3</sup>John van Geest Centre for Brain Repair, University of Cambridge

<sup>4</sup>MRC-LMB, University of Cambridge.

Olfactory dysfunction is one of the earliest and most prevalent symptoms of neurodegenerative disorders like Frontotemporal dementia, Parkinson's and Alzheimer's diseases. Odours are detected, in the first instance, by olfactory sensory neurons (OSNs) in the olfactory epithelium (OE) of the nose. OSNs are exposed to the external environment, making them accessible for biomedical studies. For these reasons, molecular analysis of the OE may reveal prodromal biomarkers of neurodegenerative brain diseases.

Here we present an olfactory analysis of a mouse model of Frontotemporal dementia and Parkinsonism linked to Chromosome 17 (FTDP-17). We find that the transgenic mice, which express a human mutant P301S *MAPT* (*Tau*) gene in all neurons, display severe olfactory deficits from an early age, even before the onset of motor or cognitive symptoms. We sequenced RNA obtained from the OE of these animals and compared it to wild-type controls. From ~17,000 genes expressed in the OE, over 2,500 genes were differentially expressed between mutants and control.

We established a reduced repertoire of genes, the "Cambridge Set", to study based on the propensity of their protein product to aggregate in neurodegenerative diseases. We show the Cambridge Set can distinguish FTDP-17 mice from a range of healthy controls. Changes in the expression of these genes in OE are very similar to changes in brain areas most affected by the pathology, like the brain stem. These changes in the OE start occurring early, at the same stage when the olfactory bulb starts showing the pathology.

This study suggests that molecular analysis of just a few genes expressed in the OE may indicate the early stages of neurodegenerative disease. They may also show the progression of the pathology. This represents a promising approach to finding prodromal biomarkers of neurodegenerative diseases. We are currently working on applying this to humans.

## **O-11: A tale of two gene sets: low and high variability in single cell RNA-seq data**

Anna Mantsoki<sup>\*</sup>, Guillaume Devailly, and Anagha Joshi

The Roslin institute, University of Edinburgh, Easter bush campus, Midlothian, EH25 9RG.

Gene expression heterogeneity contributes to development as well as disease progression. Due to technological limitations, most studies to date have focused on differences in mean expression across experimental conditions, rather than differences in gene expression variance. The advent of single cell RNA sequencing has now made it feasible to study gene expression heterogeneity and to characterise genes with low and high coefficient of variation.

We collected single cell gene expression profiles for 32 human and 39 mouse embryonic stem cells and classified genes in three groups based on their coefficient of variation (CV) across single cells (Low, Mid and High CV). We systematically characterised diverse properties distinguishing Low and High CV gene sets.

Low CV genes were enriched for cell cycle genes with their gene expression tightly controlled at transcriptional level, and showed greater conservation of sequence and expression variance across species. In contrast, High CV genes were co-expressed with other High CV genes and were enriched for bivalent (H3K4me3 and H3K27me3) marked promoters and showed a weak enrichment for embryonic development and transcription control functional categories.

Taken together, this analysis demonstrates the highly divergent characteristics of Low and High CV genes with Low CV genes representing tightly controlled genes with specific function, and High CV genes explaining bivalency at least in part.

## O-12: Single-molecule RNA sequencing in single cells.

Charles Plessy\*

RIKEN CLST, Division of Genomics Technologies, Yokohama, Japan

*Single-cell* transcriptome analysis aims to characterise exhaustively every functional RNA molecule in a cell, to accurately describe and model complex cell populations. Over the years, RIKEN has developed CAGE (*Cap Analysis Gene Expression*) to sequence the 5' ends of RNA molecules, thereby identifying transcription start sites at single molecule resolution and quantifying their activity in a single experiment.

In our recent developments for *single-cell CAGE analysis*, we modified the nanoCAGE protocol, that captures 5' ends using template-switching oligonucleotides. We added *unique molecular identifiers* to these oligonucleotides, to measure expression levels in *transcript counts*. To suppress the formation random primers dimers and rRNA signal, which waste sequencing reads, we developed "*pseudo-random*" primers, where similar sequences to rRNA and adaptors were removed. *As few as 40 different primers are enough* to prepare whole-transcriptome libraries.

To retrieve sequence information that associates novel promoters to downstream annotations, nanoCAGE libraries are designed for *paired-end sequencing*. In our original CAGEscan method, the information recovered was a single pair per transcript, and all the transcripts produced by the same promoter were aggregated into single "CAGEscan clusters". We gained *single-molecule resolution* by adding a fragmentation step using the Nextera tagmentation system. This way, PCR duplicates bearing the same unique molecular identifier produce a collection of pairs with identical 5' ends but random 3' ends, which are assembled into single-molecule "*CAGEscan fragments*" that we are validating on *Nanopore* sequencers.

We use this new protocol for the analysis of FACS-sorted single cells, and ported it to the Fluidigm C1 platform under the name "*C1 CAGE*". Our new protocol is particularly useful for studies in mammalian systems that do not benefit from a high-quality genome annotation, and is also a unique tool for the study of non-coding RNAs in human and mouse systems at the single-cell level.

### **O-13: Digital expression profiling of Purkinje neurons and dendrites in subcellular resolution**

Anton Kratz<sup>\*1</sup>, Pascal Beguin<sup>2</sup>, Stephane Poulain<sup>1</sup>, Megumi Kaneko<sup>2</sup>, Takahiko Chimura<sup>2</sup>, Ana Maria Suzuki<sup>1</sup>, Atsuko Matsunaga<sup>2</sup>, Sachi Kato<sup>1</sup>, Nicolas Bertin<sup>1</sup>, Timo Lassmann<sup>1</sup>, Rejan Vigot<sup>2</sup>, Piero Carninci<sup>1</sup>, Charles Plessy<sup>1</sup>, and Thomas Launey<sup>2</sup>

<sup>1</sup>RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Yokohama, Kanagawa, 230-0045 Japan

<sup>2</sup>RIKEN Brain Science Institute, Launey Research Unit, Wako, Saitama, 351-0198 Japan

Neuronal cells are not homogeneously distributed and subtypes are intermingled with each other as well as with non-neuronal cells such as glia and blood vessel cells. When attempting to digitally profile the expression of a specific type of neuron, retrieving the RNA only of that cell type therefore poses a considerable challenge.

In previous work, we used a technology called translating ribosome affinity purification (TRAP) to isolate the ribosome-associated transcriptome  $\diamond$  the translome. We modified it to target any cell type that can be specifically infected by a modified adeno-associated virus, and applied it to Purkinje cells (PCs) in the rat cerebellum.

We obtained quantitative expression data in single-base-pair resolution by profiling the ribosome-associated, isolated RNA using the nanoCAGE protocol. Subsequent data analysis revealed the landscape of ribosome-associated RNA of PCs in different subcellular compartments: cytoplasm and rough endoplasmatic reticulum.

In neurons, protein translation occurs not only in the soma but also distally in dendrites near or within the dendritic spines. This distal translation is thought to be regulated in response to external stimuli including long-term depression and memory formation. We have applied TRAP to Purkinje dendrites and sequenced the isolated RNA with an improved nanoCAGE protocol including a tagmentation step, to address the increased difficulty of sequencing from dendrites, which contain even less RNA than Purkinje cell soma.



## **O-14: Time-dependent host response to influenza A virus infection in Collaborative Cross founder strains and lines**

Heike Kollmus<sup>\*1</sup>, Sarah Leist<sup>1</sup>, Carolin Pilzner<sup>1</sup>, Leonie Dengler<sup>1</sup>, Robert Geffers<sup>2</sup>, Rudi Balling<sup>3</sup>, and Klaus Schughart<sup>1,4,5</sup>

<sup>1</sup>Department of Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany

<sup>2</sup>Genome Analytics, Helmholtz Centre for Infection Research, Braunschweig, Germany

<sup>3</sup>Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg

<sup>4</sup>University of Veterinary Medicine Hannover, Hannover, Germany

<sup>5</sup>University of Tennessee Health Science Center, Memphis, Tennessee, US

Influenza A virus is a zoonotic pathogen that poses a major threat to human and animal health. The course and outcome of influenza infection is influenced by viral virulence factors but also by differences in the host response. We studied the host response in the eight Collaborative Cross founder strains and several Collaborative Cross lines after influenza A H<sub>3</sub>N<sub>2</sub> infections.

The founder strains exhibited a large diversity in their response to influenza infections with respect to survival, body weight loss, hematological parameters in the blood, relative lung weight and viral load. Strain was the main factor influencing body weight loss, thus indicating that genetic variation is the major cause for the different outcomes. Analysis of survival rates and mean time to death revealed three different groups of susceptibility phenotypes: highly susceptible (A/J, CAST/EiJ, WSB/EiJ), intermediate susceptible (C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ) and highly resistant strains (NZO/HILtJ, PWK/PhJ). Viral load was largely different between susceptible and resistant strains but not between different susceptible strains. CAST/EiJ mice showed a unique phenotype. Despite very high viral loads in their lungs, they exhibited low counts of infiltrating granulocytes and showed increased numbers of macrophages in the lung. Transcriptome analyses of peripheral blood cells and lungs confirmed an aberrant immune response in CAST/EiJ. The unique phenotype of the CAST/EiJ strain may provide a novel highly valuable model to understand abnormal immune responses to infections in humans.

Our studies of the host response to influenza infections in Collaborative Cross lines revealed further variation in phenotypes which are currently being further characterized by RNAseq transcriptional profiling.

## O-15: A forward-reverse systems genetics approach to understand host-pathogen genetic interactions

Clare Smith<sup>\*1</sup>, Bibhuti Mishra<sup>1</sup>, Jarukit Long<sup>1</sup>, Megan Proulx<sup>1</sup>, Jia Yao Phuah<sup>1</sup>, Gillian Beamer<sup>2</sup>, Richard Baker<sup>1</sup>, Martin T Ferris<sup>3</sup>, Robert Williams<sup>4</sup>, and Christopher Sassetti<sup>1,5</sup>

<sup>1</sup>Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, MA, USA

<sup>2</sup>Department of Infectious Diseases and Global Health, Cummings School of Veterinary Medicine, Tufts University, MA, USA

<sup>3</sup>Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, NC, USA

<sup>4</sup>Department of Anatomy and Neurobiology, University of Tennessee Health Sciences Center, Memphis, TN, USA

<sup>5</sup>Howard Hughes Medical Institute

Infection with *Mycobacterium tuberculosis* (*Mtb*) results in a spectrum of disease, ranging from asymptomatic to lethal disease. The underlying genetic causes driving the outcome to infection are unknown and likely involve a complex interplay of both host and pathogen factors. Here, we use a dual systems genetics approach to unravel the genetic interactions between host and pathogen, exploiting diverse panels of recombinant inbred mice and genome-wide bacterial genetics.

We infected 55 Collaborative Cross (CC) lines, 19 BXD lines and parental lines of both crosses with saturated libraries of *Mtb* transposon mutants to examine interactions between mouse genotype, extent of disease, and requirements for specific bacterial virulence functions. We quantified a variety of disease phenotypes, including bacterial burden, replication dynamics, wasting, and histopathology. The disease spectrum of the CC lines exceeded that seen in parental strains and standard inbred lines. This diversity was also apparent in the specific bacterial genes important for bacterial fitness through transposon sequencing (Tnseq) analysis of libraries from each mouse line. We identified bacterial genes differentially required in each unique host genetic background during infection. Quantitative trait loci (QTL) analysis is underway to identify host polymorphisms associated with specific disease alterations and bacterial mutant fitness traits, with several QTLs so far identified underlying differential control of bacterial burden.

The combination of a classic forward genetics approach to identify host susceptibility loci in conjunction with a reverse genetic approach to assess specific bacterial fitness requirements under different host genetic backgrounds will allow dissection of the host-pathogen crosstalk driving *Mtb* disease.

## O-16: Mapping thrombosis modifier genes by bulk exome sequencing mice from a sensitized ENU mutagenesis screen

Kart Tomberg<sup>\*1</sup>, Randal J Westrick<sup>2</sup>, David R Siemieniak<sup>3,4</sup>, Guojing Zhu<sup>3</sup>, Thomas Saunders<sup>5</sup>, and David Ginsburg<sup>1,3,4,6</sup>

<sup>1</sup>Department of Human Genetics, University of Michigan

<sup>2</sup>Department of Biological Sciences, Oakland University

<sup>3</sup>Life Sciences Institute, University of Michigan

<sup>4</sup>Howard Hughes Medical Institute, University of Michigan

<sup>5</sup>Transgenic Animal Model Core Laboratory, University of Michigan

<sup>6</sup>Department of Internal Medicine, University of Michigan

Only ~10% of individuals carrying the common venous thrombosis risk factor, Factor V Leiden (FVL) will develop venous thrombosis in their lifetime. In order to identify potential FVL modifier genes, we performed a sensitized dominant ENU mutagenesis screen, based on the perinatal synthetic lethal thrombosis previously observed in mice homozygous for FVL ( $F5^{tm2Dgi}/F5^{tm2Dgi}$ ) and hemizygous for tissue factor pathway inhibitor ( $Tfpi^+/Tfpi^{tm1Gjb}$ ).

Out of ~9150 G1 (generation 1) offspring of mutagenized C57BL/6- $F5^{tm2Dgi}/F5^{tm2Dgi}$  males and unmutagenized C57BL/6- $F5^{tm2Dgi}/+$   $Tfpi^+/Tfpi^{tm1Gjb}$  females, we retrieved a total of 165 viable B6- $F5^{tm2Dgi}/F5^{tm2Dgi}$   $Tfpi^+/Tfpi^{tm1Gjb}$  progeny (rescues). As an alternative to generating a pedigree and traditionally mapping the causal mutation for each G1 rescue progeny, whole exome sequencing was applied to DNA from 103 G1 and an additional 11 G2-G8 rescue progeny to identify candidate genes that are enriched for ENU mutations. A total of 3511 likely ENU coding variants (excluding synonymous SNVs) were identified in 3009 genes. After adjusting for coding region size, the ENU-induced mutation burden for 13 genes was significantly greater than expected by chance ( $p < 0.0005$ , based on 10,000,000 permutations). Sanger sequencing validated 40 out of 41 variants within the top 13 genes. Validation of the top 6 genes (*Arl6ip5*, *C6*, *Itgb6*, *Cpn1*, *Sntg1*, *Ces3b*) is in progress, using CRISPR/CAS9 to introduce independent null alleles into mice.

Variation in these genes in humans could explain a significant portion of the incomplete penetrance and variable expressivity among patients with FVL, offer new insights into the overall regulation of hemostasis, and facilitate the development of future novel therapeutic interventions.

**O-17: A systematic functional screening approach to identify candidate genes obtained through large scale whole genome and exome sequencing of human disease cohorts.**

Peter Heutink\*, and for the International Parkinsons Disease Genomics Consortium (IPDGC)

German Center for Neurodegenerative Diseases Tuebingen.

Genome sequencing is an efficient way to identify genes related to human diseases but it also generated a major problem; How do we interpret the large volumes of sequencing data and valuable biological information from trivial data? Large scale sequencing of patients identifies hundreds to thousands of potentially damaging variants in each individual and bioinformatic analysis alone is not sufficient to identify the causal variants that can be used as the starting point for building cellular or animal models to understand the pathogenic processes of disease. We have therefore developed a systematic screening approach to prioritise candidate genes from large scale datasets for functional follow up. As proof of principle we have generated whole exome sequencing data on 1200 early onset Parkinson's Disease patients and > 2000 matched control samples. After QC we identified a total of 918,252 variants. Focussing on loss of function variants that are often associated with Autosomal Recessive Early Onset Parkinsons disease we identified 62 gene with homozygous or putative compound heterozygous loss of function variants absent in controls. In additional genetic datasets we identified further evidence for a subset of these genes and we performed systematic RNAi screens for all 62 genes using relevant assays for Parkinson's disease using three model systems; *C. elegans*; *D. melanogaster* and human neuroblastoma cell lines. As several molecular pathways have been shown to be involved in the disease process we used a variety of assays for cell viability/ alpha synuclein toxicity; mitochondrial function and morphology; intercellular translocation of PINK/Parkin. Based on our screens we have ranked our candidate genes for further follow up and have identified the molecular pathway in which each candidate gene is involved. Our approach is widely applicable and efficiently reduced the number of variants and genes to be used for in depth follow up studies.

## O-18: Natural allelic variation of interleukin 21 receptor modulates ischemic stroke outcomes

Han Kyu Lee<sup>\*1</sup>, Sehoon Keum<sup>1</sup>, Donald Lo<sup>2</sup>, and Douglas Marchuk<sup>1</sup>

<sup>1</sup>Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina, USA

<sup>2</sup>Center for Drug Discovery and Department of Neurobiology, Duke University Medical Center, Durham, North Carolina, USA

Using quantitative trait locus (QTL) linkage analysis of cerebral infarct volume after middle cerebral artery occlusion (MCAO) we previously identified a locus on distal chromosome 7 that contributes to over 50% of the variation in infarct volume. This locus shares the same location with a locus that modulates variation in collateral vessel number. Here, using interval-specific ancestral SNP haplotype analysis we fine-mapped the locus to only 12 candidate genes. One gene, interleukin 21 receptor (*Il21r*), showed a significant difference in strain-specific transcription levels. To determine whether *Il21r* modulates infarction, we examined

C57BL/6-*Il21r*<sup>tm1Wjl</sup> (*Il21r* KO) mice for their collateral vessel anatomy and cerebral infarct volume. While *Il21r* KO mice showed a moderate reduction in collateral vessel connections compared to wild-type littermates, cerebral infarct volume in *Il21r* KO mice was increased 2.3-fold. This suggests that *Il21r* has effects on both collateral vessel anatomy and innate neuroprotection. To examine the latter, we performed an *ex vivo* study of brain slices under *in vitro* oxygen deprivation (OD) and found that *Il21r* KO brain slices show an increase in OD induced neuronal cell death, showing that *Il21r* is also involved in collateral-independent neuroprotection. We determined that neuronal cell death is mediated by IL21-IL21R signaling transduction via STAT3. Interestingly, we also found a coding SNP difference in IL21R that segregates with infarct volume and collateral vessel anatomy across the strains, and determined that this sequence variation can also modulate receptor function to regulate neuronal cell death. Taken together, natural genetic variation in *Il21r* determines neuronal cell viability through modulation of receptor function, resulting in differential downstream signal transduction. Ultimately, these differences work in concert to modulate infarct volume in ischemic stroke. The identification of neuroprotective genes based on naturally occurring allelic variation will provide a path for the development of novel drug targets for ischemic stroke treatment.

## O-19: Identification of novel susceptibility genes for aggressive prostate cancer using Diversity Outbred mice

Kendra Williams<sup>1</sup>, Jonathan Andreas<sup>1</sup>, Ying Hu<sup>2</sup>, Sujata Bupp<sup>1</sup>, Daniel M Gatti<sup>3</sup>, Gary A Churchill<sup>3</sup>, and Nigel Crawford<sup>\*1</sup>

<sup>1</sup>Genetics and Molecular Biology Branch, NHGRI, National Institute of Health, Bethesda, MD 20892, USA

<sup>2</sup>Center for Biomedical Informatics and Information Technology, NCI, National Institute of Health, Rockville, MD 20850, USA

<sup>3</sup>The Jackson Laboratory, Bar Harbor, ME 04609, USA

Prostate cancer (PC) is the most commonly diagnosed non-cutaneous malignancy in men. However, <13% of cases are fatal, and more accurate means of quantifying aggressive PC risk are required to avoid over-treatment. We aimed to identify aggressive PC susceptibility genes using the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of neuroendocrine PC, which represents a particularly aggressive form of this disease. We performed quantitative trait locus mapping (QTL) in a cohort of 392 (TRAMP x J:DO) F1 males to identify genetic regions associated with susceptibility to aggressive disease susceptibility. These analyses revealed two QTLs associated with metastasis achieved genome-wide significance (Chromosome 8 [LOD=8.94], and Chromosome 15 [LOD=7.87]). For the Chromosome 8 locus, analysis of strain-specific allelic effects indicated that linkage was driven by the 129S1/SvlmJ and PWK/PhJ strains; and for the Chromosome 15 locus, linkage was driven by the PWK/PhJ, NZO/HILtJ and WSB/EiJ strains. Trait-correlation and eQTL data derived from microarray analysis of 90 (TRAMP x J:DO) F1 tumors were integrated with strain-specific SNP data to identify 24 candidate metastasis susceptibility genes. The role of these genes in aggressive human PC was investigated via an *in silico* validation that utilized human tumor gene expression datasets and a human PC genome-wide association study. This approach led to the identification of 8 novel aggressive PC susceptibility genes. Among these genes were three type II Keratin gene family members (*KRT5*, *KRT78*, and *KRT81*). Interestingly this keratin cluster, which is located on human Chromosome 12q13, has been previously implicated in germline susceptibility to PC. Our analysis demonstrates the advantage of using systems genetics approaches and mouse models to gain insights into how hereditary variation influences susceptibility to aggressive PC. Ongoing work is focusing on the functional characterization of these genes to more fully comprehend their role in susceptibility to metastasis in PC.

## O-20: Strong epistatic control of development of leishmaniasis.

Tatyana Kobets<sup>1</sup>, Yahya Sohrabi<sup>1</sup>, Matyas Sima<sup>1,2</sup>, Valeriya Volkova<sup>1</sup>, Eliska Javorkova<sup>3</sup>, Jarmila Vojtiskova<sup>1</sup>, Tereza Pokorna<sup>1</sup>, Jan Bartunek<sup>1</sup>, Alena Zajicova<sup>3</sup>, Igor Grekov<sup>1</sup>, Tatana Jarosikova<sup>4</sup>, Martina Slapnickova<sup>1</sup>, Helena Havelkova<sup>1</sup>, Milena Svobodova<sup>2</sup>, Vladimir Holan<sup>3</sup>, Peter Demant<sup>5</sup>, and Marie Lipoldova<sup>1</sup>

<sup>1</sup>Laboratory of Molecular and Cellular Immunology, Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague, Czech Republic

<sup>2</sup>Faculty of Science, Charles University, 128 44 Prague, Czech Republic

<sup>3</sup>Institute of Experimental Medicine Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague, Czech Republic

<sup>4</sup>Faculty of Biomedical Engineering, Czech Technical University in Prague, Sitna 3105, 272 01 Kladno, Czech Republic

<sup>5</sup>Roswell Park Cancer Institute, Buffalo, New York 14263, USA

Leishmaniasis, the main health problem in a number of countries, is caused by a protozoan parasite *Leishmania* that infects mononuclear phagocytes. The immune response is determined by multiple factors, including parasite characteristics and host genetics.

We developed a unique model for analysis of epistasis based on two-way interaction between genomes of *Leishmania*-resistant mouse strains, O20 and C57BL/10 (B10). Transfer of genes of O20 into background of B10 generated an inbred strain B10.O20, highly susceptible to *Leishmania*. Contrastly, transfer of B10 genes into O20 background resulted in OcB recombinant congenic strains, carrying 6.2% or 12.5% of B10 genes. OcB11 and OcB31 strains exhibited significant susceptibility. Thus, susceptible phenotypes could develop from specific combinations of genes originating from resistant strains.

After *Leishmania major* infection, B10.O20 mice evolved large lesions, high parasite numbers in skin and lymph nodes, and massive infiltration of CD11b<sup>+</sup>Gr1<sup>+</sup> cells in spleen. After stimulation with soluble *Leishmania* antigen (SLA), splenocytes of infected B10.O20 produce more Th1, Th2 and Th17 than B10 and O20, suggesting chronic inflammation with imbalance of several axes of immune response. In contrast, splenocytes of highly resistant O20 lacked response to SLA. Its intraperitoneal macrophages produced IL12, but not NO, suggesting a novel mechanism of resistance. As B10.O20 carries only 4.2% of O20-derived genes, it offers an opportunity to study this strong epistasis.

We also studied susceptibility to *L. major* in 15 OcB RC strains. Using F<sub>2</sub> hybrids, derived from OcB43 (an OcB31 substrain), we detected that loci on Chromosomes 3 and 15 controlled parasite numbers in liver. Lesion was controlled by the interaction between loci on Chromosomes 2 and 3.

Combining detailed genetic analysis of these models with analysis of immunological and pathological parameters of infected mice and gene expression studies will provide a powerful tool to describe different mechanisms of resistance and susceptibility. Grant GACR 13-41002P.

## O-21: Metabolic regulation by the MECP2 transcriptional repressor complex points to new therapeutic targets in Rett syndrome

Stephanie M Kyle<sup>\*1,2</sup>, and Monica J Justice<sup>1</sup>

<sup>1</sup>The Hospital for Sick Children, Toronto, Ontario, Canada

<sup>2</sup>Baylor College of Medicine, Houston, Texas, USA

Metabolic dysregulation can lead to downstream pathogenesis in nearly all tissues and organ systems. In recent decades, a large body of data has implicated metabolic perturbations in neurological development and degeneration. In particular, dysregulation of cholesterol trafficking and biosynthesis are responsible for the onset of Neimann-Pick type C and Smith-Lemli Opitz syndrome, respectively. Furthermore, Fragile X syndrome, Alzheimer, Parkinson, and Huntington diseases have all been linked to aberrant cholesterol homeostasis. Rett syndrome (RTT) is a progressive neurodevelopmental disorder of females primarily caused by mutations in the X-linked gene encoding methyl-CpG-binding protein 2 (*MECP2*). To identify pathways in disease pathology for therapeutic intervention, we carried out a dominant random mutagenesis suppressor screen in *Mecp2* null mice. One suppressor identifies a stop codon mutation in a rate-limiting enzyme in cholesterol biosynthesis, which ameliorates RTT-like symptoms and increases longevity in *Mecp2* null mice by altering cholesterol homeostasis. Although RTT has been classically labeled a neurological disorder, these studies suggest that a metabolic component contributes to pathology. Here we show that *Mecp2* deletion induces hyperlipidemia, fatty liver, and metabolic syndrome in mice. These metabolic phenotypes are strikingly similar to that in mice with a liver-specific knockout of histone deacetylase 3 (*Hdac3*), a potent regulator of lipogenesis and cholesterol biosynthesis. Consistently, we show that MECP2 and HDAC3 work in complex to suppress expression of the cholesterol enzyme identified in our screen, as well as other genes of the cholesterol and *de novo* lipogenesis pathways. Our data suggest a novel metabolic component in RTT, arising from loss of interaction between MECP2 and HDAC3. Concurrently, liver-specific deletion of *Mecp2* is deleterious enough to cause fatty liver through aberrant lipogenic gene transcription. Our ongoing studies point to additional metabolic pathways that are prime targets in the pursuit of preventing morbidities associated with Rett syndrome.



## O-22: Mouse Models of Human Diaphragmatic Birth Defects: The mesothelium performs a fundamental role in proper formation of the diaphragm

Nicole Paris, and Kate Ackerman\*

Department of Pediatrics and Biomedical Genetics, University of Rochester Medical Center, Rochester, NY, 14642

Understanding genes and signaling pathways critical for normal diaphragm development is important for understanding human congenital defects of the diaphragm. Since many of the mouse genetic knock out models are embryonic lethal, we have worked to identify Cre Recombinase mouse lines useful for creating conditional deletion of genes required for diaphragm development. We have characterized an inducible *Wt1* *CreERT2* mouse line (STOCK-*Wt1<sup>tm2(cre/ERT2)Wtp</sup>*) and used it to understand whether  $\beta$ -catenin (CTNNB1) plays a critical role in diaphragm development. We have also used these models to investigate the role for the WT1+ mesothelium in normal diaphragm signaling and development.

We performed fate mapping of *Wt1* expressing cells to the diaphragm after timed injections of tamoxifen (STOCK-*Wt1<sup>tm2(cre/ERT2)Wtp</sup>* x STOCK-*Gt(ROSA)26Sor<sup>tm1Sho</sup>*), and we performed tissue specific, temporally regulated CTNNB1 loss-of-function and gain-of-function experiments with analysis of embryonic diaphragm development. These models primarily altered signaling in the diaphragmatic mesothelium. Loss of WT1 resulted in decreased WNT signaling and *Ctnnb1* expression during a critical time period of development (E9.5-E12.5). Conditional loss of *Ctnnb1* with *Wt1<sup>tm2(cre/ERT2)Wtp</sup>* mice caused large bilateral posterior diaphragm defects similar to the phenotype of *Wt1* mutants while CTNNB1 activation rescued this phenotype. Although apoptosis and proliferation were affected, these genetic manipulations resulted in large changes in mesenchymal differentiation.

The diaphragmatic mesothelium functions to regulate normal diaphragm development. CTNNB1 is critical for diaphragm development during a defined window of time, and the mechanism of the diaphragm defects of the *Wt1* and *Ctnnb1* mutants involve disruption of basic cellular processes.

## O-23: Genomic responses in mouse models greatly mimic human inflammatory diseases

Tsuyoshi Miyakawa\*

Fujita Health University

The use of mice as animal models has long been considered essential in modern biomedical research, but the role of mouse models in research was challenged by a recent report that genomic responses in mouse models poorly mimic human inflammatory diseases (Seok et al., PNAS, 2013). We have been investigating the molecular basis of psychiatric disorders using gene expression analyses in mice models of the disorders. By applying the same analysis methods we have been using, we reevaluated the same gene expression datasets used in the study by Seok et al. Contrary to the previous findings, the gene expression levels in the mouse models showed extraordinarily significant correlations with those of the human conditions (Spearman's rank correlation coefficient: 0.43-0.68; genes changed in the same direction: 77-93%;  $P = 6.5 \times 10^{-11}$  to  $1.2 \times 10^{-35}$ ). Moreover, meta-analysis of those datasets revealed a number of pathways/bigroups commonly regulated by multiple conditions in humans and mice. These findings demonstrate that gene expression patterns in mouse models closely recapitulate those in human inflammatory conditions and strongly argue for the utility of mice as animal models of human disorders.

In this talk, I will introduce the debates ignited by the studies and also discuss the general significance of mice models of human diseases.

## O-24: Bipartite structure of the inactive mouse X Chromosome

Xinxian Deng<sup>1,7</sup>, Wenxiu Ma<sup>2,7</sup>, Vijay Ramani<sup>2,7</sup>, Andrew Hill<sup>7</sup>, Fan Yang<sup>1,7</sup>, Ferhat Ay<sup>2,7</sup>, Joel B Berletch<sup>1,7</sup>, Carl Anthony Blau<sup>3,4,7</sup>, Jay Shendure<sup>2,7</sup>, Zhijun Duan<sup>3,4,7</sup>, William S Noble<sup>2,5,7</sup>, and Christine M Disteche<sup>\*1,6,7</sup>

<sup>1</sup>Department of Pathology

<sup>2</sup>Department of Genome Sciences

<sup>3</sup>Institute for Stem Cell and Regenerative Medicine

<sup>4</sup>Division of Hematology

<sup>5</sup>Department of Computer Science and Engineering

<sup>6</sup>Department of Medicine

<sup>7</sup>University of Washington, Seattle, Washington, USA

We analyzed allele-specific chromatin contacts by a new Hi-C assay that uses DNase I for chromatin fragmentation to evaluate structural changes associated with X inactivation and imprinting in mouse F1 hybrid systems in which alleles can be distinguished based on single nucleotide polymorphisms. Both *in vivo* (brain) and *in vitro* (Patski cells) the two X Chromosomes have strikingly different 3D configurations. Two superdomains of frequent long-range intrachromosomal contacts separated by a hinge region are specifically observed on the inactive X Chromosome. Such a bipartite 3D organization has been also reported in human lymphoblastoid cells. We found that the genomic content of the superdomains is rearranged between human and mouse, but that part of the hinge region is conserved and located near the lncRNA *Dxz4/DXZ4* locus that binds CTCF on the inactive X. In mouse, the hinge region also contains a minisatellite Ds-TR adjacent to a promoter with strong CTCF binding. Both *Dxz4* and Ds-TR bind nucleophosmin and are enriched in nucleolus-associated chromatin, suggesting anchoring to the nucleolus. Genes that escape X inactivation and regions enriched in CTCF or RNA polymerase are preferentially located on the periphery of the inactive X while LINE1 elements are preferentially on the interior. Genes subject to silencing exhibit fewer detectable short-range intrachromosomal contacts than escape genes. This transcription-coupled pattern is also evident for imprinted genes, in which more chromatin contacts are detected on the expressed allele, suggesting greater constraint on the organization of expressed genomic regions.

## O-25: Genetic and dietary effects on gametic selection at fertilization

Ghunwa Nakouzi<sup>1,2</sup>, Delphine Carouge<sup>3</sup>, Sabine Schaefer<sup>3</sup>, and Joseph Nadeau<sup>\*3</sup>

<sup>1</sup>Center for Human Genetics, University Hospitals of Cleveland

<sup>2</sup>Department of Genetics, Case Western Reserve University School of Medicine, Cleveland

<sup>3</sup>Pacific Northwest Diabetes Research Institute, Seattle

Detecting Mendel's Law of Segregation is usually based on gametes combining randomly at fertilization, independent of their genetic constitution. With rare exception such as t-haplotypes in mice, the X-chromosome in crosses with *Mus spretus*, and SD in *Drosophila*, Mendelian expectations hold. However, recent studies reveal new exceptions based on seemingly common dietary and genetic effects. Dietary folate supplementation reduces the incidence and severity of neural tube defects (NTDs) in humans and mouse models. In some models, dietary supplementation leads to highly non-random genotypic ratios that are usually interpreted as folate-induced embryonic lethality of mutant homozygotes and heterozygotes. In addition, litter sizes are not reduced and resorption rates are not increased, suggesting that lethality does not account for the unusual genotypic ratios. Instead, deviations from Mendelian expectations in intercrosses but not backcrosses suggest preferential fertilization between particular eggs and sperm. The second example involves the Deadend1 gene (*Dnd1*, miRNA control), Apobec1 complementation factor (*A1cf*), the Apobec1 cytidine deaminase gene (*Apobec1*, RNA editing) and other genes involved in RNA biology, which have heritable epigenetic effects on susceptibility to testicular germ cell tumors (TGCTs). Mutations in these genes, either alone or in combination, also lead to strong deviations from Mendelian expectations in intercrosses but not backcrosses. Again, neither litter sizes nor resorption rates are affected. Thus both folate supplementation as well as mutations in *Dnd1*, *A1cf*, *Apobec1* and related genes lead to a strong bias for fertilization with wild-type rather than mutant alleles. We propose that anomalies in polyamine metabolism mediates the fertilization bias with folate supplementation of NTD mutants. Both the dietary and genetic discoveries must be more fully characterized and mechanisms identified because of their implications for our understanding of inheritance and fertilization as well as for public health policies concerning dietary supplementations to prevent common birth defects.

## O-26: Epigenetic inheritance of diet induced obesity and diabetes via oocyte and sperm

Peter Huypens<sup>1</sup>, Daniela Dyckhoff<sup>1</sup>, Moya Wu<sup>1</sup>, Steffen Sass<sup>3</sup>, Susan Marschall<sup>1</sup>, Fabian Theis<sup>3,4</sup>, Martin Hrabe de Angelis<sup>1,2,5</sup>, and Johannes Beckers<sup>\*1,2,5</sup>

<sup>1</sup>Institute of Experimental Genetics and German Mouse Clinic, Helmholtz Zentrum Munchen GmbH, 85764 Neuherberg, Germany

<sup>2</sup>German Center for Diabetes Research (DZD), 85764 Neuherberg, Germany

<sup>3</sup>Institute of Computational Biology, Helmholtz Zentrum Munchen GmbH, 85764 Neuherberg, Germany

<sup>4</sup>Technische Universitat Munchen, Department of Mathematics, 85747 Garching, Germany

<sup>5</sup>Technische Universitat Munchen, Chair of Experimental Genetics, 85354 Freising, Germany

The inheritance of epigenetic information across generations has been controversial in mammals. Some reports provided initial evidence that a paternal high fat diet may propagate obesity and glucose intolerance in offspring, but potential confounders such as molecular factors present in seminal fluid or paternal-induced alterations in maternal care were not ruled out in these studies. We show in mice that a parental high fat diet renders offspring derived via *in vitro* fertilization (F<sub>1</sub>) more susceptible to develop excessive overweight and type 2 diabetes (T2D) in a gender and parent-of-origin specific mode. Female, but not male, offspring from obese parents became significantly more obese during a HFD challenge than female offspring from lean parents. Body weight trajectories and distribution patterns of individual body weights in female offspring from one obese and one lean parent demonstrate that paternal and maternal germline propagate obesity in a roughly equitable and additive fashion, but likely different mode of action. In contrast, a more deteriorated state of HFD-induced insulin resistance was observed in both F<sub>1</sub> genders, albeit predominantly inherited via the maternal germline. Analyses of transcriptome and methylome signatures in gametes as well as the analysis of parental systemic factors that may contribute to the soma-to-germline information transfer are currently ongoing. We report for the first time epigenetic inheritance of an acquired metabolic disorder via mammalian oocytes and sperms excluding confounding factors. Such an epigenetic mode of inheritance may contribute to the observed pandemic increase in obesity and T2D prevalence rates, especially in an environment where nutrition is abundant.

## O-27: Exploring regulatory networks through omics data

S Sethi<sup>1</sup>, S Greenaway<sup>1</sup>, S Kumar<sup>1</sup>, J Fernandez<sup>2</sup>, M Simon<sup>1</sup>, and Ann-Marie Mallon<sup>1</sup>

<sup>1</sup>MRC Harwell, Harwell Science and Innovation Campus, Harwell OX11 0RD, UK

<sup>2</sup>The Wellcome Trust Centre for Human Genetics, University of Oxford, OX3 7BN, UK

Transcriptional regulation involves a complex network of transcription factors (TFs) binding to DNA regulatory elements to control gene expression. In order to understand transcriptional regulation, we require the genomic locations of regulatory elements, the identity of factors that bind them, and the genes they target. ChIP-seq has been widely used to identify *in vivo* transcription factor binding sites (TFBSs), promoters and enhancers across the genome. DNase-Seq is used to identify DNASE1 hypersensitive sites (DHSs) across the genome indicating regions which have open chromatin and are accessible to DNA-binding proteins. RNA-Seq identifies co-expressed genes. My aim is to explore the publicly available data in ENCODE to study transcriptional regulation and to develop novel methods to analyse the phenodeviants at Mammalian Genetics Unit (MGU).

Here I present a novel method to identify known and novel TFBSs using DHSs. Using ChIP-seq data of various histone modifications from mouse ENCODE, we performed Chromatin segmentation in 22 tissues. This systematically characterized the mouse genome into regions of promoters and enhancers. Using this method, we observe that on average promoters, enhancers and insulators cover 1.25%, 4.5%, 0.8% of the mouse genome respectively. Overlying this data with DHSs, we discovered that TFBSs are highly enriched in DHSs as compared to promoter and enhancer regions suggesting DNase1 hypersensitivity data could be used as an alternative method for discovering enriched regulatory motifs.

We further implemented this method on RNA-Seq data from different mouse models of human diseases and found novel direct and indirect regulatory interactions. Future applications involve extending this model to include a phylogenetic module complexity analysis to detect conserved co-occurring TFBSs patterns within cis-regulatory modules.

## **O-28: Retinal cell therapy using iPS cells**

Masayo Takahashi<sup>\*</sup>

RIKEN Center for Developmental Biology

The first in man application of iPS-derived cells started in September 2014 targeted the retinal disease called age-related macular degeneration (AMD). The grafted iPS-derived retinal pigment epithelial (RPE) cell sheet is survived well and good in color, that means no immune rejection occurred without immune suppression. Her visual acuity is stable, compare to the past history of deterioration even with multiple anti-VEGF injections. Primary endpoint, the safety was achieved at one year point.

We evaluated plasmid remnant & gene alteration using WGS, epigenetic characteristics and purity using single cell RT-PCR other than our original quality control (QC). From these experiences, we think we should do both but should distinguish between basic research and regulatory science in order to promote regenerative medicine promptly.

Since autologous transplantation is time consuming and the cost is high, it is necessary for making standard treatment to prepare allogeneic transplantation using HLA three loci homozygous iPS cell lines (iPS cell stocks) as well as autologous transplantation. It is known that RPE cells suppress the activation of T-cells, so that RPE cells appeared most suitable for such kind of allogeneic transplantation. We confirmed in vitro and in vivo that human iPS-derived RPE cells also have such function. It is possible that the rejection is considerably small by using the iPS-RPE cell with matched three loci of HLA.

## O-29: CRISPR/Cas9 genome editing in rodents: *In vivo* and *in vitro* applications

Marie-Christine Birling<sup>\*1</sup>, Philippe Andre<sup>1</sup>, Sylvie Jacquot<sup>1</sup>, Laurence Schaeffer<sup>1</sup>, Marie Wattenhofer-Donze<sup>1</sup>, Guillaume Pavlovic<sup>1</sup>, and Yann Herault<sup>1,2</sup>

<sup>1</sup>Institut Clinique de la Souris, PHENOMIN, CNRS UMR7104, INSERM U964, Université de Strasbourg, 1 rue Laurent Fries BP 10142 Parc d'Innovation 67404 Illkirch, France

<sup>2</sup>Institut de Genetique Biologie Moleculaire et Cellulaire (IGBMC), CNRS, INSERM, Université de Strasbourg, UMR7104, UMR964, Illkirch, France

The CRISPR/Cas9 system allows to generate insertions, deletions, duplications or substitutions at specific sites in rodents by simple pronuclear injection of the Cas9 mRNA or protein, one or more specific guide mRNA and a DNA template for specific modifications (when a specific modification is required). In many cases, this technology abolishes the need of embryonic stem cells.

We have obtained deletions through non-homologous end joining (NHEJ) with efficiencies up to 70% in both mouse and rat. We are currently working on improving the use of CRISPR/Cas9 for integrating mutations by homology-directed repair in order to be able to generate quicker and cost effective customized rodent models. We are also applying the CRISPR/Cas9 technology to the generation of mouse model that will be phenotyped in the IMPC (<http://www.mousephenotype.org/>). KO alleles are generated by deletion of one or more critical exon(s) in genes which are not currently available as targeted ES cells. Even more exciting, the CRISPR/Cas technology allows the generation, in an impressively fast way, of deletion/duplication of big genomic region (3-4 months versus to 3-4 years with the standard TAMERE route). We have easily achieved the deletion/ duplication of a 24 megabases genomic DNA fragment in rat.

We are also using successfully the CRISPR/Cas9 system *in vitro* in ES cells to improve the targeting efficiency for projects which failed previously. We have currently recovered 5 projects for which we were not able to obtain any targeted ES cells (in some case we screened previously more than 1500 ES cells) by standard electroporation. The simple addition of a plasmid expressing the Cas9 and a guide RNA recognizing the site of insertion of the selection cassette has dramatically improved the homologous recombination rate.

A few cases of both *in vivo* and *in vitro* experiments will be presented and discussed.



### **O-30: CRISPR/Cas9-mediated plasmid knock-in and replacement of genomic region with single stranded oligonucleotides in rodents**

Kazuto Yoshimi<sup>\*1</sup>, Takehito Kaneko<sup>2</sup>, Tsuyoshi Koide<sup>1</sup>, and Tomoji Mashimo<sup>3</sup>

<sup>1</sup>Mouse Genomics Resource Laboratory, National Institute of Genetics

<sup>2</sup>Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University

<sup>3</sup>Institute of Experimental Animal Sciences, Graduate School of Medicine, Osaka University

Genome editing technologies such as ZFN, TALEN and CRISPR/Cas9 have enabled generating genetically modified animals within the past few years. Development of an efficient knock-in (KI) technology will facilitate easy and flexible genome engineering to introduce precise mutations or genetic modifications at any target sites of any cells from any strains and species.

We have reported the efficient generation of several types of KI rats using single-stranded oligodeoxyribonucleotides (ssODNs), such as SNP substitution, small DNA fragment insertion, and DNA fragment elimination of a 7kb retrotransposon element (Yoshimi K *et al.*, Nat Commun 2014). It is easy to design and synthesize 80-160 base ssODNs donors, while the limitation of ssODN-mediated KI is the maximum length of the insert DNA, which is less than 100bp.

To generate the targeted KI with longer DNA fragments such as GFP reporter genes, we used CRISPR/Cas9 as "scissors" to cut at targeted sites in genome DNA and the plasmid DNA, and ssODNs as "paste" to ligate the ends of the cut sites. Co-microinjection of Cas9 mRNA, two gRNAs, two ssODNs and CAG-GFP donor plasmids into fertilized eggs in rats and mice resulted in pups carrying a CAG-GFP KI at the targeted *Gt(Rosa26)Sor* loci. The methods enabled not only efficient KI of plasmids without homology arms, including a 200kb bacterial artificial chromosome, but also the replacement of rat genes to human-specific genes.

These gene KI technologies are essentially applicable to any targeted site with any donor vector as it is in any species, which can provide the generation of genetically humanized models in a variety of species for understanding the mechanisms of human disease and physiological function.

## O-31: CRISPR-Driven Replacement of a Mouse Tumor Suppressor with 25-kbp of the Orthologous Human Gene

Tiffany Leidy-Davis, Kai Cheng, Meghan Schultz, Leslie Goodwin, Jiayuan Shi, Judy Morgan, and David Bergstrom\*

Genetic Resource Science, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609 USA

Our interests lie in humanizing specific regions of the mouse genome using large segments of human DNA with extents of 10s to 100s of kilobase pairs. In the recent past, we achieved the targeted replacement of an 18-kbp segment of the mouse genome using a classic embryonic stem cell approach coupled with traditional recombineering and dual (G418/puromycin) selection.

To speed and simplify the humanization of mouse genes, here, we report the CRISPR-driven replacement (humanization) of an 18-kilobase pair (kbp) segment of a mouse tumor suppressor gene with an orthologous, disease-associated, 25-kbp segment of the corresponding human gene. Four *Cas9*/sgRNAs were designed to introduce redundant double-stranded breaks at each end of the 18-kbp mouse region. The four guides, along with a specially designed donor vector containing the 25-kbp segment of human sequence, were then introduced into C57BL/6 zygotes by microinjection.

Among 82 G<sub>0</sub> founder animals, three were positive for the human sequence. Despite the possibility of mosaicism, loss-of-allele assays demonstrate a "hemizygote-like" copy number loss of the 18-kbp mouse segment in two of the G<sub>0</sub> animals, and a "homozygote-like" copy number loss of the 18-kbp segment in the third. To date, the third G<sub>0</sub> animal has given rise to progeny carrying either the 25-kbp humanized segment or an 18-kbp deletion but not both, consistent with this G<sub>0</sub> animal carrying the 25-kbp humanized segment in *trans* to an 18-kbp deletion.

The new technique has numerous advantages including ♦

- 1) Obviating the need for antibiotic selection of embryonic stem cells,
- 2) Avoiding the recombinase-mediated excision of selection cassettes,
- 3) Expanding the physical size of CRISPR-driven knock-ins and gene replacements to  $\geq$  25-kbp,
- 4) Opening multiple strains and species to long range DNA modification.

The presentation details the specifics of our project, provides a brief overview of the technique, and describes progress to date.

### **O-32: The Estimation of Selective Effects Using Large Scale Population Data Identifies Genes Required For Normal Mammalian Development.**

CA Cassa<sup>1</sup>, DM Jordan<sup>1</sup>, DJ Balick<sup>1</sup>, DP Nusinow<sup>1</sup>, SR Sunyaev<sup>1</sup>, and David Beier<sup>\*2</sup>

<sup>1</sup>Division of Genetics, Harvard Medical School/Brigham and Women's Hospital, Boston, MA, USA

<sup>2</sup>Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute/Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA

The generation of large amounts of sequence data from various populations has enabled unbiased queries into the significance and potential consequences of DNA sequence variation in disease. We used the genome-wide distribution of expected and observed nonsense mutations in 60,706 patient exomes without severe Mendelian disorders from Exome Aggregation Consortium (ExAC) to estimate the strength of heterozygote selection for every human gene. We were specifically interested in those with high heterozygote selection; i.e., the cohort of genes for which one does not find nonsense mutations. Given that the sequenced population was viable, one may hypothesize that haploinsufficiency for these genes is not compatible with survival. Genes of this type are already well known; in humans they are often associated with defects in embryonic development. Notably, heterozygosity for many of these genes is tolerated in mice; while homozygous mutants have defects that recapitulate features of the human syndrome. This is fortuitous in that it enables the experimental characterization of molecular consequences of the mutation.

Indeed, the cohort of genes we identified with high heterozygote selection included many well-known human developmental disease genes. Importantly, our approach was predictive for genes with developmental effects, as the strength of heterozygote selection was highly correlated with the likelihood of recessive lethality for a set of 767 KOMP/EUCOMM alleles characterized by the Wellcome Trust/Sanger phenotyping program. We further characterized the gene set using a text-mining analysis; remarkably, a very large fraction of the high heterozygote selection cohort have little functional annotation. This provides a potentially robust opportunity for identifying novel genes with critical developmental roles. Further, given the empirical evidence that the high heterozygote selection gene set includes many that are causal for congenital defect syndromes, it is likely that analysis of uncharacterized genes in this cohort will be informative with respect to human disease.

### **O-33: The landscape of replication associated mutations in the human and mouse germlines**

Lana Talmane<sup>1</sup>, Martin Reijns<sup>1</sup>, Harriet Kemp<sup>1</sup>, Robert Young<sup>1</sup>, James Ding<sup>1</sup>, Sophie Marion de Proce<sup>1</sup>, Ian Adams<sup>1</sup>, Rod Mitchell<sup>2</sup>, Wendy Bickmore<sup>1</sup>, Andrew Jackson<sup>1</sup>, and Martin Taylor<sup>\*1</sup>

<sup>1</sup>MRC Human Genetics Unit, MRC IGMM, University of Edinburgh, UK

<sup>2</sup>MRC Centre for Reproductive Health, University of Edinburgh, UK

Genetic mutations provide the raw material for evolution, they are responsible for heritable disease and driving the development of cancer. We have shown that the binding of chromatin and regulatory proteins to DNA can interfere with replication and lead to regions with locally elevated mutation rates. Mechanistically this process appears to involve the trapping of DNA polymerase alpha synthesised DNA in the fully replicated genome; a process we have explored with a novel method, EmRiboSeq, that tracks replicative polymerase activity *in vivo*. Extending this work we have measured the patterns of chromatin accessibility and protein binding specifically in the mammalian germline and related it to the distribution of polymorphism and mutation, to reveal the terrain of replication associated mutations in mice and humans. This provides a means of adjusting neutral substitution rate estimates for fine-scale mutation rate fluctuation when identifying regions of selective constraint. We also identify likely hotspots of paternal lineage mutations within functional regulatory sites.

### O-34: Paradoxical evolution of a large segmental duplication in mouse

Andrew P Morgan<sup>\*1</sup>, Rachel C McMullan<sup>1</sup>, Amelia M-F Clayshulte<sup>1</sup>, Timothy A Bell<sup>1</sup>, Grace Clark<sup>1</sup>, James M Holt<sup>2</sup>, Leonard McMillan<sup>2</sup>, and Fernando Pardo-Manuel de Villena<sup>1</sup>

<sup>1</sup>Department of Genetics, University of North Carolina, Chapel Hill, NC, USA

<sup>2</sup>Department of Computer Science, University of North Carolina, Chapel Hill, NC, USA

Gene duplication and loss are major sources of genetic polymorphism in populations and important forces shaping the evolution of genome content and organization. We have reconstructed the evolutionary history of a 125 kbp duplicated segment, *R2d*, in the laboratory mouse (*Mus musculus*). The sequence is absent from the mouse reference genome but controls its own meiotic segregation in *cis* in a copy-number dependent manner. After an initial duplication event ~2 Mya in the common ancestor of *M. musculus* and *M. spretus*, the resulting paralogs (*R2d1*, *R2d2*) were subject to genetic drift, inter-locus gene conversion, further duplication and loss. We show that the *R2d2* locus remains unstable: its copy number ranges from 0 to more than 80 in laboratory and wild mice sampled from around the globe, and mutation rate for new CNVs in laboratory populations exceeds 1% per generation. *R2d* encompasses a single protein-coding gene, *Cvc22*, which is expressed from all its paralogs and rapidly-evolving in rodents. Yet at the nucleotide level, sequence diversity in noncoding regions of *R2d2* (but not *R2d1*) is significantly reduced relative to the genome-wide average. I will discuss implications for interpretation of sequence divergence at other duplicated loci, and present evidence for an effect of parental age on *de novo* mutation rate for large CNVs at *R2d2*.

### **O-35: The frequent evolutionary birth and death of functional promoters in mouse and human**

Robert Young<sup>\*1</sup>, Yoshihide Hayashizaki<sup>2</sup>, Robin Andersson<sup>3</sup>, Albin Sandelin<sup>3</sup>, Hideya Kawaji<sup>2</sup>, Masayoshi Itoh<sup>2</sup>, Timo Lassmann<sup>4</sup>, Piero Carninci<sup>4</sup>, The FANTOM consortium<sup>4</sup>, Wendy Bickmore<sup>1</sup>, Alistair Forrest<sup>4,5</sup>, and Martin Taylor<sup>1</sup>

<sup>1</sup>MRC Human Genetics Unit, MRC Institute for Genetics and Molecular Medicine, University of Edinburgh, Crewe Road, Edinburgh, EH4 2XU, UK

<sup>2</sup>RIKEN Preventive Medicine and Diagnosis Innovation Program, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

<sup>3</sup>Department of Biology & Biotech Research and Innovation Centre, Copenhagen University, Ole Maaloes Vej 5, Copenhagen N, Denmark

<sup>4</sup>RIKEN Center for Life Science Technologies (Division of Genomic Technologies), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045, Japan

<sup>5</sup>Systems Biology and Genomics, Harry Perkins Institute of Medical Research, QEII Medical Centre, Nedlands, WA 6009, Australia

Promoters are central to the regulation of gene expression. Changes in gene regulation are thought to underlie much of the adaptive diversification between species and phenotypic variation within populations. In contrast to earlier work emphasizing the importance of enhancer evolution and subtle sequence changes at promoters, we show that dramatic changes such as the complete gain and loss (collectively turnover) of functional promoters are common. Using quantitative measures of transcription initiation in both humans and mice across 52 matched tissues we discriminate promoter sequence gains from losses and resolve the lineage of changes. We also identify expression divergence and functional turnover between orthologous promoters, finding only the latter is associated with local sequence changes. Promoter turnover has occurred at the majority (>56%) of protein-coding genes since humans and mice diverged. Tissue-restricted promoters are the most evolutionarily volatile where retrotransposition is an important, but not the sole source of innovation. There is considerable heterogeneity of turnover rates between promoters in different tissues, but the consistency of these in both lineages suggests the same biological systems are similarly inclined to transcriptional rewiring. The genes affected by promoter turnover show evidence of adaptive evolution. In mice, promoters are primarily lost through deletion of the promoter containing sequence; whereas in humans, many promoters appear to be gradually decaying with weak transcriptional output and relaxed selective constraint. Our results suggest that promoter gain and loss is an important process in the evolutionary rewiring of gene regulation and may be a significant source of phenotypic diversification.

### **O-36: Elucidation of the Underlying Mechanism of the Induction of Pluripotency Genes by SAHA-Conjugated Pyrrole-Imidazole Polyamides by Computational Genomic Analysis**

Jason Lin<sup>\*1</sup>, Hiroyuki Yoda<sup>1</sup>, Kiriko Hiraoka<sup>1</sup>, Takahiro Inoue<sup>1</sup>, Sakthisri Krishnamurthy<sup>1</sup>, Takayoshi Watanabe<sup>1</sup>, Atsushi Takatori<sup>1</sup>, Nobuko Koshikawa<sup>1</sup>, Seiya Imoto<sup>3</sup>, Satoru Miyano<sup>3</sup>, Hiroshi Sugiyama<sup>2</sup>, and Hiroki Nagase<sup>1</sup>

<sup>1</sup>Laboratory of Cancer Genetics, Chiba Cancer Center Research Institute

<sup>2</sup>Department of Chemistry, Graduate School of Science, Kyoto University

<sup>3</sup>Laboratory of DNA Information Analysis, Institute of Medical Science, University of Tokyo.

We have been actively developing highly sequence-specific pyrrole-imidazole DNA minor-groove binding polyamides as molecular switches of various biological triggers. Those polyamides demonstrate remarkable specificity *in situ* to DNA sequences, and further functionalization of this class of molecules provides a novel and effective approach in the development of molecular cancer therapy *in vivo*. Some of our recent advances include the development of a class of small molecules called SAHA-PIP comprising of histone deacetylase inhibitor SAHA and DNA binding pyrrole-imidazole polyamide capable of genome-wide epigenetic reprogramming. In mouse embryonic fibroblasts, we found that SAHA-PIP could induce multiple pluripotency-associated genes such as Rex1 and Cdh1; similar results were also observed in human dermal fibroblast cells. The ability for SAHA-PIP to turn the highly conserved genetic machinery of pluripotency greatly demonstrate the scientific and therapeutic potential of pyrrole-imidazole polyamides as an inhibitors delivery system for histone modifications.

Additionally, comparative genomic studies of SAHA-PIP binding site predictions, combined with microarray experiments, may further reveal the mechanistic insights on the critical genes involved in the epigenetic reprogramming process. We are currently utilizing various statistical and computational methods to understand the effect of SAHA-PIP on the induction of pluripotency, cellular reprogramming and oncogenesis. Our previous reports on the exceptional effect of SAHA-PIP have led us to explore the possibility of chromatin opening, gene activation and disrupting transcription factor binding events in the genome with a library of SAHA-conjugated pyrrole-imidazole polyamides. From comparing findings from the human and mouse genome, we may then understand the underlying biology behind cellular reprogramming and epigenetic regulation via SAHA-PIP.

## O-37: Capybara genome sequencing offers deeper insights into rodent evolution

Isaac Adeyemi Babarinde<sup>\*1,2</sup>, and Naruya Saitou<sup>1,2</sup>

<sup>1</sup>National Institute of Genetics, Mishima, Japan

<sup>2</sup>Graduate University for Advanced Studies, Mishima, Japan

Rodents are the mammalian order with the highest number of species and ecological success. Mouse and rat are two representative rodent species that have been extensively studied as model species. These two species are taxonomically close, belonging to the Murinae subfamily. However, rodents have about thirty families classified into three morphologically different clades. Are the genomic and evolutionary features of mouse and rats true representatives of the rodent order? In this study, we focused on evolutionary rate as an evolutionary feature. Using mouse and rat as representatives, higher evolutionary rate has been reported in rodents. The higher evolutionary rate has been attributed to shorter generation interval. Since generation interval is known to positively correlate with body size, we decided to sequence the whole genome of capybara, the largest living rodent species. Here, we report the first whole genome draft sequence of capybara. The assembled genome was about 2.4 Gbp with an average depth of 15 $\times$ . Capybara's genomic GC content of ~40% is comparable to those of other rodent species. Using a combination of bioinformatics and statistical approaches, we extracted genomic regions with high quality. The extracted high-quality regions were more than 1.8 Gbp and the average high-quality read depth was 8 $\times$ . Using the high-quality regions, heterozygosity was estimated to be 0.0022. We performed comparative genomics with previously reported mammalian genomes. Using various estimates of evolutionary rates, we report some aspects of rodent evolution that are hitherto hidden. This study lays a foundation in understanding the genomics of capybara and offers deeper insights into rodent evolution.



## O-38: The Short Story of a Long Tale

Janan Eppig\*

The Jackson Laboratory, Bar Harbor, ME 04609, USA

The mission of the Mouse Genome Informatics (MGI, [www.informatics.jax.org](http://www.informatics.jax.org)) resource is to provide integrated genetic, genomic, and biological data about the laboratory mouse to facilitate the study of human health and disease. This mission is unchanged since its inception. But the dramatic changes in the scientific landscape ushered in by molecular biology, the sequencing of the human and mouse genomes, and the rapid advances in computer technology mean that the MGI of today is radically different than the MGI of 25+ years ago.

In this talk, I will

- ◆ begin at the beginning, in how I came to bioinformatics in the first place; and the initial collaborations that formed MGI.
- ◆ show major change-points in MGI's evolution and growth.
- ◆ fast forward to describe new changes to the user experience at the MGI web site (release date late October 2015).

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### **O-39: Informatics for the International Mouse Phenotyping Consortium- a Platform for Phenotypic and Translational Discovery**

Terrence F Meehan<sup>\*1</sup>, and On Behalf of the Mouse Informatics Phenotyping Infrastructure -MPI<sup>2,2,3</sup>

<sup>1</sup>European Molecular Biology Laboratory - European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SD, UK MRC

<sup>2</sup>Mammalian Genetics Unit, MRC Harwell, Harwell Science and Innovation Campus, Harwell OX11 0RD, UK

<sup>3</sup>Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK

The International Mouse Phenotyping Consortium (IMPC) is delivering new insights into biological mechanisms and human disease by generating and characterizing thousands of knockout mouse strains for genes with little or no known biological function. For each mouse, thousands of data points are collected according to a standardized, broad-based phenotyping pipeline and archived centrally within the IMPC-Data Coordinating Centre. Dedicated 'data wranglers' ensure quality of the data and an automated statistical analysis pipeline identifies mouse strains with abnormal phenotypes. As of August 2015, phenotype data from 2000 knockout strains is freely available and is providing global views into fertility, sexual dimorphism and the genetic mechanisms underlying a wide range of phenotype traits. Potential disease models are identified by orthologous gene and orthologous phenotype features and are used in prioritizing gene variant candidates for human genetic diseases. In addition, a dedicated pipeline is assessing dysmorphology in embryonic lethal strains by using state-of-the-art imaging analysis technologies. Users can freely access all data via an intuitive web portal that allows biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research. The community is invited to explore and provide feedback as we build this rich resource at: **[www.mousephenotype.org](http://www.mousephenotype.org)**

## **O-40: Systemic metabolic phenotyping in the German Mouse Clinic in search of new mouse models for metabolic disorders**

Jan Rozman<sup>\*1,6</sup>, Robert Brommage<sup>1</sup>, Birgit Rathkolb<sup>1,2</sup>, Manuela Oestereicher<sup>1</sup>, Helmut Fuchs<sup>1</sup>, Valerie Gailus-Durner<sup>1</sup>, Martin Klingenspor<sup>3,4</sup>, Eckhard Wolf<sup>2,6</sup>, and Martin Hrabe de Angelis<sup>1,5,6</sup>

<sup>1</sup>German Mouse Clinic, Institute of Experimental Genetics, Helmholtz Zentrum Muenchen, German Research Center for Environmental Health, Germany

<sup>2</sup>Institute of Molecular Animal Breeding and Biotechnology, Gene Center, Ludwig-Maximilians-Universitaet Muenchen, Germany

<sup>3</sup>Molecular Nutritional Medicine, Else Kroener-Fresenius Center, Technische Universitaet Muenchen, Germany

<sup>4</sup>ZIEL Center for Nutrition and Food Sciences, Technische Universitaet Muenchen, Germany

<sup>5</sup>Chair of Experimental Genetics, Center of Life and Food Sciences Weihenstephan, Technische Universitaet Muenchen, Germany

<sup>6</sup>German Center for Diabetes Research (DZD)

The annotation of gene functions related to metabolic disorders such as obesity and type 2 diabetes is a key area in international large scale mouse phenotyping efforts. The German Mouse Clinic (GMC) is member of the International Mouse Phenotyping Consortium (IMPC) contributing to the global effort to generate a knockout mouse model for every protein-coding gene and provide phenotyping data to relate gene functions to human disease.

Several IMPC phenotyping assays address metabolic functions: clinical blood chemistry, insulin blood levels, indirect calorimetry, intraperitoneal glucose tolerance test, body composition analysis, combined SHIRPA and dysmorphology, as well as gross and histopathology.

We have particular interest in the areas regulation of energy metabolism and glucose homeostasis. Mechanisms involved in primary suppression or upregulation of metabolic rate are of special interest for the understanding of why energy balance regulation is impaired in obese or cachectic patients. We developed and applied data analysis tools that identify primary effects of single gene knock outs on metabolic regulation. In a preliminary survey of 99 mutant lines (>2,000 mice) from the GMC, 6% of the mutant lines were hypometabolic whereas 10% were hypermetabolic when adjusted for body mass differences.

Impaired glucose tolerance and hyperglycemia as diagnostic criteria for a pre-diabetic or diabetic state can be evaluated with a glucose tolerance test after overnight food deprivation. We evaluated the IMPC standard operating procedure and developed an easy and intuitive way of identifying genes related to dysfunctional glucose homeostasis. In a first step, evaluating fasting blood glucose values as the ratio of mutant to wildtype levels for >1200 lines identified outliers with either low or high fasting glycemia.

These initial steps in data analysis and functional annotation of genes indicate that the IMPC data resource offers a unique source of disease oriented phenotype information open to the scientific community.

## O-41: Genetic architecture of behavior in an advanced intercross line of mice

Natalia M. Gonzales<sup>\*1</sup>, Jungkyun Seo<sup>1</sup>, Shyam Gopalakrishnan<sup>3</sup>, and Abraham A. Palmer<sup>1,2</sup>

<sup>1</sup>Department of Human Genetics, University of Chicago, Chicago, IL 60637

<sup>2</sup>Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL 60637

<sup>3</sup>Natural History Museum of Denmark, Copenhagen University, Copenhagen, Denmark

Mice are one of the primary model organisms used to study behavior. We are conducting a genome-wide association study of behavior in a LG/J x SM/J advanced intercross line (AIL) of mice (Aap:LG,SM-G50-56, derived from Jmc:LG,SM-G33). An AIL is generated by crossing two inbred strains for multiple generations and offers greater precision for mapping quantitative trait loci (QTL) than traditional genetic crosses.

We measured prepulse inhibition, locomotor activity, and a number of other traits in ~1,100 male and female AILs (Aap:LG,SM-G50-56). We genotyped ~2,000 AILs spanning over 12 generations (Aap:LG,SM-G34;G39-43;G50-56) using genotyping by sequencing (GBS) and used RNA-sequencing to map gene expression QTL (eQTL) in brain tissue from ~250 mice (Aap:LG,SM-G50-56).

Here we present preliminary eQTL identified in the hippocampus, striatum and prefrontal cortex and describe how we are integrating these data with QTL to identify genes underlying variation in locomotor activity and prepulse inhibition. We are also building an online database that includes all of the genotypes, phenotypes and RNA-sequencing data we have collected from the LG/J x SM/J AIL since G34. We will provide an example of how this resource is being used to replicate and refine QTL that we identified in previous studies.

Our results demonstrate that AIL mice can be used to identify genes that are involved in behavioral traits at a fraction of the cost and effort of a human mapping study. Integrating genotype, phenotype and gene expression data is a powerful approach that will accelerate the process of gene identification and provide insight into the biology of complex traits.

## **O-42: The hidden layer of regulatory RNA in mammalian genome biology**

John Mattick\*

Garvan Institute of Medical Research, Sydney

It appears that the genomic programming of mammalian biology has been misunderstood for the past 50 years, because of the assumption that most genetic information is transacted by proteins. The mammalian genome contains only ~20,000 protein-coding genes, similar in number and with largely orthologous functions as those in other animals, including simple nematodes. On the other hand, the extent of non-protein-coding DNA increases with increasing developmental and cognitive complexity, reaching 98.5% in humans. Moreover, high throughput analyses have shown that the majority of the mammalian genome is dynamically transcribed during differentiation and development to produce tens if not hundreds of thousands of short and long non-protein-coding RNAs that show highly specific expression patterns and subcellular locations. Increasing numbers of these RNAs are being shown to have functions at many different levels of gene expression, including translational control and the guidance of epigenetic processes that underpin development, physiological adaptation, brain function and transgenerational communication, augmented by the superimposition of plasticity via RNA editing, RNA modification and retrotransposon mobilization. This suggests that there is a massive hidden layer of RNA-based information flux in mammalian genome biology and that the simple protein-centric operator-repressor model of 'gene regulation' derived from studies of bacteria is incorrect in highly organized and spatially specialized multicellular organisms. This in turn requires reassessment of the nature, hierarchies and scaling of the regulatory systems that control the evolution, 4-dimensional assembly and cognitive capacities of animals.

## **O-43: *In vivo* profiling of the promoter- and enhancer landscape of inflammatory bowel disease**

Mette Boyd<sup>1</sup>, Morana Vitezic<sup>1</sup>, Jette Bornholdt<sup>1</sup>, Malte Thodberg<sup>1</sup>, Kristoffer Vitting-Seerup<sup>1</sup>, Thilde Bagger Terkelsen<sup>1</sup>, Mehmet Coskun<sup>1,2</sup>, Robin Andersson<sup>1</sup>, Jesper Troelsen<sup>3</sup>, Ole Haagen Nielsen<sup>2</sup>, Jacob Bjerrum<sup>2</sup>, and Albin Sandelin<sup>\*1</sup>

<sup>1</sup>Department of Biology and BRIC, University of Copenhagen, Copenhagen, Denmark

<sup>2</sup>Department of Gastroenterology, Medical Section, University of Copenhagen, Herlev Hospital, Herlev, Denmark

<sup>3</sup>Department of Science, Systems and Models, Roskilde University, Roskilde, Denmark

Coordinated gene regulation is essential for all aspects of cell biology, including development, differentiation and disease. Characterization of enhancers and promoters in disease has been difficult due to the lack of genome-wide methods suitable for the analysis of small tissue samples. Therefore, we know little about the regulation of genes in disease, and its variation between patients. Related to this, 85% of protein-coding genes show heritable variation in expression due to variance in gene regulation. Thus, localization of promoters and enhancers genome-wide within patient material is important for disease biology and genetics.

Because both promoter and enhancers are transcribed, they can be detected by RNA sequencing. Utilizing this, we have profiled promoter and enhancer usage in patients suffering from inflammatory bowel disease (IBD). IBD is a complex group of chronic inflammatory conditions in the gut. Crohn's disease (CD) and Ulcerative Colitis (UC) are the two principal subtypes. Correct treatment depends on accurate sub-type diagnosis, which is challenging and expensive.

To this end, we have profiled the descending colon of 110 human subjects, stratified into disease subtypes and controls. We identified a promoter set that with high accuracy can distinguish the shared inflammatory response, and UC- or CD-specific profiles. The set included annotated promoters, alternative promoters and promoters of novel long non-coding RNAs.

Moreover, we identified over 20,000 enhancer regions that are active in these samples, where subsets are induced in general inflammation or in UC/CD specifically. These enhancers are often linked to known and novel IBD-induced genes, suggesting that they are important in the pathogenesis. Connected to this, IBD-associated SNPs were highly enriched in these regulatory regions, enabling subsequent identification of casual regulatory mutations.

To our knowledge, this is the first comprehensive profiling of enhancers and promoters in large patient cohorts for any disease.

## O-44: Enhancers lead waves of coordinated transcription in transitioning mammalian cells

Erik Arner<sup>\*1</sup>, Carsten O Daub<sup>1</sup>, Kristoffer Vitting-Seerup<sup>2</sup>, Robin Andersson<sup>2</sup>, Kim M Summers<sup>3</sup>, Christine Wells<sup>4</sup>, David A Hume<sup>3</sup>, Alistair Forrest<sup>1</sup>, Albin Sandelin<sup>2</sup>, Piero Carninci<sup>1</sup>, and Yoshihide Hayashizaki<sup>5</sup>

<sup>1</sup>Div. of Genomic Technologies, Center for Life Science Technologies, RIKEN, Japan

<sup>2</sup>University of Copenhagen, Denmark

<sup>3</sup>Roslin Institute, University of Edinburgh, United Kingdom

<sup>4</sup>Institute for Infection Immunity and Inflammation, University of Glasgow, United Kingdom

<sup>5</sup>RIKEN Preventive Medicine and Diagnosis Innovation Program, Japan

Cellular differentiation requires coordinated induction of genes, facilitated by dynamic regulation of promoters and enhancers by transcription factors. Exploiting the fact that active promoters and enhancers are transcribed, and that genome-scale 5'RACE (CAGE) detects transcription start sites (TSS) including the bidirectional TSS characteristic of active enhancers, we simultaneously measured the activity of promoters and enhancers in 19 human and 14 mouse time courses covering a wide range of cell types and biological stimuli, to dissect the relationship between dynamic changes in mRNA and eRNA. The time courses included stem cells (embryonic, induced pluripotent, trophoblastic and mesenchymal stem cells) and committed progenitors undergoing terminal differentiation towards mesodermal, endodermal and ectodermal fates, as well as fully differentiated primary cells and cell lines responding to stimuli (growth factors and pathogens). Enhancer RNAs dominated the earliest expression responses, followed by mRNAs encoding transcription factors and then by other transcripts. Binding sites for key lineage transcription factors were simultaneously over-represented in enhancers and promoters active in each cellular system. Our data support a highly generalizable model in which enhancer transcription is the earliest event in successive waves of transcriptional change during cellular differentiation or activation, and the multitude of biological systems studied suggests that this phenomenon is a general feature of mammalian transcriptional regulation. This challenges previous models which suggested that linked enhancers and promoters are co-expressed over time.

## O-45: Network architecture of microRNA regulation in mammalian cells

Michiel De Hoon<sup>1,3</sup>, Derek De Rie<sup>1,4</sup>, Tanvir Alam<sup>9</sup>, Erik Arner<sup>1,3</sup>, Peter Arner<sup>24</sup>, Haitham Ashoor<sup>9</sup>, Gaby Astrom<sup>24</sup>, Magda Babina<sup>19</sup>, Nicolas Bertin<sup>1,3,28</sup>, Maxwell Burroughs<sup>8</sup>, Carsten O Daub<sup>1,3</sup>, Michael Detmar<sup>7</sup>, Alexandre Fort<sup>1,3</sup>, Dan Goldowitz<sup>13</sup>, Sven Guhl<sup>19</sup>, Jayson Harshbarger<sup>1,3</sup>, Akira Hasegawa<sup>1,3</sup>, Kosuke Hashimoto<sup>1,3</sup>, Hideya Kawaji<sup>1,2,3</sup>, Peter Klinken<sup>20</sup>, Timo Lassmann<sup>1,3,6</sup>, Charles Lecellier<sup>29</sup>, Weonju Lee<sup>11</sup>, Marina Lizio<sup>1,3</sup>, Vsevolod Makeev<sup>31</sup>, Anthony Mathelier<sup>30</sup>, Yulia Medvedeva<sup>25,26,27</sup>, Chris Mungall<sup>16</sup>, Shohei Noma<sup>1,3</sup>, Mitsuhiro Ohshima<sup>17</sup>, Helena Persson<sup>15</sup>, Filip Roudnicky<sup>7</sup>, Pal Saetrom<sup>12</sup>, Jessica Severin<sup>1,3</sup>, Kim M Summers<sup>10</sup>, Hiroshi Tarui<sup>1,3</sup>, Kristoffer Vitting-Seerup<sup>5</sup>, Christine Wells<sup>23</sup>, Louise Winteringham<sup>20</sup>, Yoko Yamaguchi<sup>19</sup>, The FANTOM consortium, Albin Sandelin<sup>5</sup>, Michael Rehli<sup>21,22</sup>, Yoshihide Hayashizaki<sup>2,3</sup>, Piero Carninci<sup>1,3</sup>, and Alistair Forrest<sup>14</sup>

- <sup>1</sup>RIKEN Center for Life Science Technologies (Division of Genomic Technologies), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 Japan
- <sup>2</sup>RIKEN Preventive Medicine and Diagnosis Innovation Program, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 Japan
- <sup>3</sup>RIKEN Omics Science Center (OSC), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 Japan [RIKEN Omics Science Center ceased to exist as of April 1st 2013 due to RIKEN reorganization]
- <sup>4</sup>Centre for Integrative Bioinformatics (IBIVU), VU University Amsterdam, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands
- <sup>5</sup>The Bioinformatics Centre, Department of Biology and Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Ole Maaloes Vej 5, DK 2200 Copenhagen, Denmark
- <sup>6</sup>Telethon Kids Institute, 100 Roberts Road, Subiaco, WA 6008, Australia
- <sup>7</sup>Swiss Federal Institute of Technology (ETH) Zurich, Institute of Pharmaceutical Sciences, HCI H390, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland
- <sup>8</sup>National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, 8600 Rockville Pike, Bethesda, MD 20894, USA
- <sup>9</sup>Computational Bioscience Research Center, Computer, Electrical and Mathematical Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia
- <sup>10</sup>The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush, Edinburgh, Midlothian EH25 9RG, UK
- <sup>11</sup>Department of Dermatology, Kyungpook National University School of Medicine, 130 Dongdeok-ro Jung-gu, Daegu 700-721, South Korea
- <sup>12</sup>Department of Computer and Information Science, Norwegian University of Science and Technology, N-7489 Trondheim, Norway
- <sup>13</sup>Department of Medical Genetics, University of British Columbia, 950 West 28th Avenue, Vancouver, BC V5Z 4H4, Canada
- <sup>14</sup>Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, the University of Western Australia, Nedlands, Western Australia, Australia
- <sup>15</sup>Division of Oncology and Pathology, Department of Clinical Sciences, Lund University, Medicon Village, building 404:B3, Scheelevaegen 2, SE-223 81 Lund, Sweden
- <sup>16</sup>Genomics Division, Lawrence Berkeley National Laboratory, 84Ro1, 1 Cyclotron Road, Berkeley, California 94720, USA
- <sup>17</sup>Department of Biochemistry, Ohu University School of Pharmaceutical Sciences Misumido 31-1, Tomitamachi, Koriyama, Fukushima 963-8611, Japan
- <sup>18</sup>Department of Biochemistry, Nihon University School of Dentistry, 1-8-13, Kanda-Surugadai, Chiyoda-ku, Tokyo 101-8310, Japan
- <sup>19</sup>Department of Dermatology and Allergy, Charite Campus Mitte, Universitaetsmedizin Berlin, Chariteplatz 1, 10117 Berlin, Germany
- <sup>20</sup>Harry Perkins Institute of Medical Research, and the Centre for Medical Research, University of Western Australia, QQ Block, QEII Medical Centre, Nedlands, Perth, Western Australia 6009, Australia
- <sup>21</sup>Department of Internal Medicine III, University Hospital Regensburg, Regensburg, Germany
- <sup>22</sup>Regensburg Centre for Interventional Immunology (RCI), Regensburg, Germany
- <sup>23</sup>Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland, Brisbane St Lucia, Queensland 4072, Australia
- <sup>24</sup>Department of Medicine, Karolinska Institutet at Karolinska University Hospital, Huddinge, SE-141 86 Huddinge, Sweden
- <sup>25</sup>Josep Carreras Leukaemia Research Institute (LJC), Muntaner 383, 3r 2a, 08021 Barcelona, Spain
- <sup>26</sup>Vavilov Institute of General Genetics, Russian Academy of Science, Gubkina str., 119333 Moscow, Russia
- <sup>27</sup>Bioengineering Centre, Russian Academy of Science, pr. 60-letiya Oktyabrya, 7-1, 117312, Moscow, Russia
- <sup>28</sup>Cancer Science Institute of Singapore, National University of Singapore, 14 Medical Dr, 117599 Singapore
- <sup>29</sup>Institute of Molecular Genetics of Montpellier, CNRS-UMR 5535, 1919 Route de Mende, 34293 Montpellier, Cedex 5, France
- <sup>30</sup>Centre for Molecular Medicine and Therapeutics (CMMT), University of British Columbia | Child & Family Research Institute, 980 West 28th Avenue, Room 3109 | V5Z 4H4, Vancouver, BC, Canada
- <sup>31</sup>Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov str. 32, Moscow 119991, Russia.

The regulatory network governing cellular behavior includes both transcription factors and miRNAs as key regulators. To understand the role of miRNAs in the integrated network architecture of cellular regulation, we analyze deep sequencing data of paired small RNA and Cap Analysis of Gene Expression (CAGE) libraries across a wide range of cell types, in particular human primary cells. We found that microRNAs can be divided into two classes based on their expression pattern: Cell type specific miRNAs that are highly expressed only in a few cell types, and ubiquitous miRNAs that are expressed in most cell types but depleted in particular cell types. Ubiquitous miRNAs preferentially target genes expressed in the cell types in which the miRNA is depleted, and may play a role in preventing inappropriate activation of specific transcriptional programs. In contrast, cell type specific miRNAs also target genes co-expressed with the miRNA, and may play a role in buffering gene expression. We find that promoter sequences of miRNAs are highly conserved and that miRNA expression is primarily regulated at the transcriptional level, with ubiquitous miRNAs preferentially regulated by repressors, and cell type specific miRNAs both by activators and repressors.



## O-46: Regulated mobilization of Retrotransposable elements in cell identity, reprogramming and disease

Beatrice B Bodega<sup>1,2</sup>, Francesco Della Valle<sup>3</sup>, Loqmane Seridi<sup>3</sup>, Yanal Gosheh<sup>2</sup>, Federica Marasca<sup>3</sup>, Gregorio Alanis-Lobato<sup>3</sup>, Manjula Thimma<sup>4</sup>, Sara Pinosio<sup>5</sup>, Valentina Saccone<sup>5</sup>, Sara Consalvi<sup>6</sup>, Jenny Martone<sup>6</sup>, Valentina Cazzella<sup>7</sup>, Marina Mora<sup>6</sup>, Irene Bozzoni<sup>5</sup>, Per Lorenzo Puri<sup>4</sup>, Michele Morgante<sup>3</sup>, Timothy Ravasi<sup>2</sup>, and Valerio Orlando<sup>2,3</sup>

<sup>1</sup>Istituto Nazionale di Genetica Molecolare (INGM) Romeo and Enrica Invernizzi, Genome Biology Unit, Milan, Italy

<sup>2</sup>IRCSS Fondazione Santa Lucia, Epigenetics and Genome Reprogramming, Rome, Italy

<sup>3</sup>KAUST Environmental Epigenetics Research Program, Biological Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology, Kingdom of Saudi Arabia

<sup>4</sup>Institute of Applied Genomics, Udine, Italy

<sup>5</sup>IRCCS Fondazione Santa Lucia, Pharmacology and Epigenetics, Rome, Italy

<sup>6</sup>Department of Biology and Biotechnology Charles Darwin, Sapienza University of Rome, and Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Italy

<sup>7</sup>Division of Neuromuscular Diseases and Neuroimmunology, Istituto Nazionale Neurologico C. Besta, Milano, Italy

The rapid progress of genomics technologies, in particular high-resolution Cap Analysis Gene Expression (CAGE) for transcriptome analysis and whole genome sequencing (WGS) have been unveiling global regulatory features in the eukaryotic genome by which transposable elements (TEs) would provide substantial contribution to the genetic and epigenetic cell program. Whether the global TEs distribution is random or follows regulated developmental programs remains to be elucidated. To this aim we investigated L1 dynamics during differentiation of human primary muscle cells, finding that skeletal myogenesis supports a MyoD- and HDAC2/Dystrophin-NOS1 dependent activity of this class of repetitive elements resulting in the acquisition of L1 elements specifically at expressed skeletal muscle gene loci. We found that this phenomenon is impaired during differentiation of muscle cells derived from patients affected by Duchenne muscular Dystrophy (DMD), being HDAC2 aberrantly recruited at L1 promoter and their transcription repressed. Pharmacological rescue of DMD phenotype by HDAC inhibitors or gene therapy approach by exon-skipping is accompanied by normal L1 expression and CNV both in C57BL/6J-*Dmd*<sup>mdx</sup> model mice and in human DMD primary muscle cells. We found that during differentiation L1 and other TEs are transcribed following a characteristic profile made of successive waves of activation that closely matches enhancer elements and myogenic program. Interestingly this profile is flat in DMD cells as consequence of global defect in chromatin acetylation. In another work we investigated L1 dynamics in somatic cell reprogramming finding that L1 mobilization is required also for efficient reprogramming. We propose that L1 repetitive elements mobilization is an integral regulatory part of developmental and cell specialization programs and their epigenetic deregulation a key trait in loss of cell identity and disease.

## **O-47: Modeling Psychiatric/Neurological disorders using iPS cell technologies and transgenic non-human primates.**

Hideyuki Okano\*

Dept of Physiology, Keio University Graduate School of Medicine, Japan

What makes the investigation of human psychiatric/psychiatric disorders so difficult? This could be attributed to the following reasons 1) Diseases model mice do not always recapitulate the pathophysiology of human diseases, 2) It is extremely difficult to investigate what is taking place *in vivo* at the onset of the disease due to the low accessibility to the pathological foci in the brain, and 3) The responsible neuronal circuits for the phenotype are not identified. In order to overcome these difficulties, we took advantage of iPS cell technologies and transgenic non-human primates for modeling human psychiatric/psychiatric disorders. So far, we have established iPS cells from the patients of about 40 human psychiatric/psychiatric disorders and characterized their pathophysiology. For example, in collaboration with the group of RIKEN BSI and University of Tokyo, we established iPS cells from the schizophrenia patients containing 22q11 deletions (Bundo et al., *Neuron*, 2014). Interestingly, we found that the copy number of a retrotransposon, long interspersed nuclear element-1 (L1), was increased in neurons induced from iPS cells from schizophrenia patients containing 22q11 deletions, indicating that hyperactive retrotransposition of L1 in neurons triggered by genetic risk factors may contribute to the susceptibility and pathophysiology of schizophrenia.

Furthermore, for faithfully modeling the human psychiatric/psychiatric disorders *in vivo*, we developed transgenic non-human primates (common marmosets) with germline transmission (Sasaki et al., *Nature*, 2009). In the present talk, we also wish to mention our recent data of generation of common marmoset transgenic models of neurodegenerative diseases, including Parkinson disease, Alzheimer disease and ALS. Furthermore, we could generate knock-out technologies of common marmoset using genome editing technologies for the generation of transgenic marmoset model of autism and psychiatric disorders.

At the end, I will mention about Brain Mapping Projects in Japan, in which investigation of common marmoset brains plays key roles.

## O-48: GENCODE: revealing transcriptional complexity in Human and Mouse

Mark Thomas<sup>\*</sup>, Jennifer Harrow, and GENCODE Consortium

Wellcome Trust Sanger Institute, United Kingdom

GENCODE is now the default human gene set in both the UCSC and Ensembl genome browsers, combining computational and manual genome annotation approaches. With an emphasis on alternative splicing, our current release (v23) contains 198,619 transcripts based on EST and mRNA evidence from 19,797 protein coding and 15,931 long non-coding genes. The increasing availability of next-generation sequencing data from RNAseq, CAGEseq and PolyAseq, allows us to define transcribed regions with ever increasing accuracy; adding to the transcriptional complexity of the genome. Understanding this transcriptional complexity is important for the study of disease, especially now that CRISPR-Cas9 technologies are driving a genome-editing revolution. Identifying functional transcripts is particularly important, so as to differentiate them from transcripts arising via stochastic events or spliceosomal errors. The function of most protein coding transcripts is evident from the encoded protein, whereas the function of long non-coding transcripts is more difficult to determine. In an effort to improve our functional understanding of transcripts, we are using advances in ribosome profiling and mass spectrometry to assess the coding potential of transcripts. Combining these approaches not only highlights how differences in the precise TSS can influence the translational start of proteins, but has also allowed us to identify entirely novel proteins. The same approaches are currently being used to improve annotation of the mouse genome, which remains an important model for studying disease. As a result, we now have regular releases of the mouse GENCODE gene set for the C57BL/6 reference genome. This annotation will be extended to other laboratory mouse strains with *de-novo* genome assemblies through collaboration with the Sanger Mouse Genomes project.

## O-49: FANTOM6: Functional elucidation of lncRNA

Jay W Shin<sup>\*</sup>, Michiel De Hoon, and Piero Carninci

RIKEN Yokohama - Center for Life Science Technologies, Division of Genomics Technologies

FANTOM is an international research consortium initially to assign functional annotations to the full-length cDNAs that were collected during the Mouse Encyclopedia Project at RIKEN ([fantom.gsc.riken.jp](http://fantom.gsc.riken.jp)). FANTOM has since developed and expanded over time to encompass the fields of human transcriptomics. The recent FANTOM has unraveled a myriad of transcriptome diversity across 400 human cell types where many were considered non-coding RNAs (FANTOM5 consortium; *Nature* 2014). Cellular diversity of these lncRNA and their dynamic interplays between enhancers and promoters suggest much more investigation is needed to elucidate their function. In the 6th edition of FANTOM, we aim to broadly generate a reference set of profiles for multiple human cell types using the latest next generation sequencing (NGS) technologies followed by large-scale perturbation of lncRNAs and infer a molecular phenotype via CAGE analyses; in parallel, we will functionally characterize and classify lncRNAs using technologies complementary to CAGE. Here, I will introduce the next FANTOM and reveal our current pipeline to build our unique collection of functional lncRNA atlas.

## O-50: Multiple mouse reference genomes and strain specific gene annotations

Benedict Paten<sup>1</sup>, Mario Stanke<sup>2</sup>, Son Pham<sup>3</sup>, David Thybert<sup>4</sup>, Laura G. Reinholdt<sup>5</sup>, Jennifer Harrow<sup>6</sup>, Kerstin Howe<sup>6</sup>, David Adams<sup>6</sup>, and Thomas Keane<sup>\*6</sup>

<sup>1</sup>University of California, Santa Cruz

<sup>2</sup>University of Greifswald

<sup>3</sup>University of California, San Diego

<sup>4</sup>European Bioinformatics Institute, Hinxton, UK

<sup>5</sup>Jackson Laboratory, Bar Harbor, ME

<sup>6</sup>Wellcome Trust Sanger Institute, Hinxton, UK

The generation of the mouse reference genome sequence from the C57BL/6J strain was a major milestone in recent mouse genetics history. It has enabled a whole set of new applications and technologies. In phase 2 of the Mouse Genomes Project, we are producing accurate *de novo* genome sequences and strain specific gene annotation for 16 strains (129S1/SvImJ, A/J, ARK/J, BALB/cJ, C3H/HeJ, C57BL/6NJ, CAST/EiJ, CBA/J, DBA/2J, FVB/NJ, LP/J, NOD/ShiLtJ, NZO/HILtJ, PWK/PhJ, SPRET/EiJ, and WSB/EiJ) from a mixture of short and long range illumina libraries, optical maps, and third generation sequencing. In 2015 we produced the first set of pseudo chromosome sequences for each strain. In a collaboration with Dovetail Genomics, we produced near chromosome length *de novo* scaffolds for CAST/EiJ, PWK/PhJ, and SPRET/EiJ. Extensive QC and validation is being carried out in preparation for full public release. Work is underway to produce strain specific gene sets by a combined comparative gene prediction approach using the C57BL/6J genes with strain specific evidence such as RNA-Seq and PacBio cDNA sequencing. Our initial analysis on Chromosome 11 has revealed new gene structures at small and large scale, not present in the existing Gencode C57BL/6J gene set. In complex and highly polymorphic complex loci such as the major urinary proteins (*Mups*), *H2*, and *Irg*, we have identified new allelic forms of these genes, gene shuffling, large translocations, and incorporation of open reading frames (ORFs) from other parts of the genome. We are working with the UCSC Genome Browser group to develop a comparative mouse strains browser to visualise the genomic structural differences in conjunction with the strain specific gene predictions.

## O-51: A survey of genome rearrangements in human evolution

Martin Frith\*

National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan

Genomes evolve in part by rearrangements, such as inversions and translocations, which reorder the DNA code in a drastic and perhaps irreversible way. Despite great interest, there has been no comprehensive survey of rearrangements in human or mammal evolution, beyond simple ones like inversions.

Here we survey all types of human rearrangements that have occurred since the last common ancestor with chimpanzee. We use a recent algorithm to align the human and chimp genomes, which finds orthologies more accurately and lacks bias against micro-rearrangements [Frith and Kawaguchi 2015]. We systematically infer rearrangement events from the alignments, and classify them based on the minimum number of DNA breaks needed to produce them. We sub-classify them by topology (inversion, interchromosomal translocation, etc.) We exclude chimp-specific rearrangements (including misassemblies) by checking their presence in human-orangutan alignments.

We find 291 rearrangements, including 193 inversions, but also complex ones requiring at least five DNA breaks. Many of the rearrangements seem to have arisen by DNA shattering into multiple fragments, which then re-joined in random order and orientation, with some fragments being lost. This resembles damage from radiation (e.g. natural radon gas or cosmic rays) repaired by non-homologous end-joining. It also resembles "chromothripsis", observed recently in cancer and other diseases. Some inversions were caused by shattering, others by recombination between inverted repeats, and it is usually easy to tell these apart. We reconstruct some shattering-and-joining events at the single-base level.

Most rearrangements do not overlap known exons. However, one interesting inversion swapped the upstream halves of two divergently-transcribed chymotrypsinogens, *CTRB1* and *CTRB2*. This was accompanied by gene conversion, so that the upstream halves of both genes resemble the ancestral *CTRB2*.

This study reveals what kinds of rearrangements have occurred, and the likely mechanisms. We plan to check these rearrangements in ancient hominin (e.g. Neanderthal) DNA.

## **O-52: An experimental approach to elucidate enigmatic isochore evolution by using ENU mutagenesis**

Satoshi Oota<sup>\*1</sup>, Ryutaro Fukumura<sup>2</sup>, Naruya Saitou<sup>3</sup>, and Yoichi Gondo<sup>2</sup>

<sup>1</sup>Bioresource Information Division, RIKEN BioResource Center

<sup>2</sup>Mutagenesis and Genomics Team, RIKEN BioResource Center

<sup>3</sup>Division of Population Genetics, National Institute of Genetics

Historical events in the mouse genome are obviously key factors to determine various characteristics of mouse strains. For making use of the mouse resource, therefore, it is crucially important to elucidate the genome evolution. One of the big issues of the mammalian genome evolution is the "isochore" problem: a queer spatial structure of the genome in GC content. So far, no coherent explanations were achieved due to contradictory observations against the proposed models: i.e., selection, biased gene conversion, and mutation bias models. Today, the typical approach to study evolution is the "backward" analysis by using extant molecular data to infer the past by extrapolation. In the isochore evolutionary study, however, this conventional approach is insufficient because we have at least two kinds of issues to be considered: (1) the evolutionary signal of interest might be eroded by unknown evolutionary process; (2) we need to handle non-coding regions, to which it is difficult to apply conventional evolutionary models. Especially, (1) is a critical problem because subtle information in non-coding sequences may be easily worn out even during short-term evolution. To overcome those problems, we took advantage of ENU mutagenesis as a tool for the experimental evolution, accelerating evolutionary rate to make it possible to observe ongoing evolution through a large number of de novo mutations. We found bidirectional (diverged) mutation pressures that support the legendary Sueoka's mutation bias theory. Our finding also has potential to explain the enigmatic isochore evolution.

### **O-53: Post-translational mechanisms buffer protein abundance against transcriptional variation.**

Steven Munger<sup>\*1</sup>, Joel Chick<sup>2</sup>, Petr Simecek<sup>1</sup>, Edward Huttlin<sup>2</sup>, Kwangbom Choi<sup>1</sup>, Daniel M Gatti<sup>1</sup>, Narayanan Raghupathy<sup>1</sup>, Karen L Svenson<sup>1</sup>, Steven Gygi<sup>2</sup>, and Gary A Churchill<sup>1</sup>

<sup>1</sup>The Jackson Laboratory, Bar Harbor, Maine USA

<sup>2</sup>Harvard Medical School, Cambridge, Massachusetts USA

Recent studies have reported low correlation between protein and mRNA abundance, which has led to the prediction of widespread buffering of protein expression against variation in mRNA levels. However, the relative importance of transcriptional and post-transcriptional modes of protein regulation remains poorly understood. Our study builds on earlier observations, integrating new techniques in mass spectrometry to expand the breadth of protein quantification, new methods to accurately quantitate transcript abundance in RNA-seq data, and new mouse models with extensive genetic variation to perturb mRNA and protein expression. We identified 1728 proteins that are regulated by local genetic variation; for 80% of these proteins, the causal variant acts proximally on transcript abundance, and consequently there is high correlation between protein and transcript abundance. Further, we identified 1362 proteins that are regulated by distant genetic variation. In stark contrast to local associations, nearly all distant loci appear to act on target protein abundance independent of their cognate mRNA via unknown post-transcriptional mechanism(s) - aka protein buffering. We applied a novel mediation approach to identify causal regulatory proteins and transcripts underlying these distant loci. Our analysis revealed an extensive network of protein-protein interactions that act to achieve stoichiometric balance of functionally related enzymes and subunits of multimeric complexes, and moreover provides new insights into the specific mechanisms of protein buffering.



## O-54: Analysis of energy demands during lactation in mouse models

Jerry Wei<sup>1,2</sup>, Walter Olea<sup>2</sup>, Louise Hadsell<sup>2</sup>, Peter Thomson<sup>1</sup>, Darryl Hadsell<sup>2,3</sup>, and Peter Williamson<sup>\*1</sup>

<sup>1</sup>Faculty of Veterinary Science, The University of Sydney, Sydney, NSW 2006, Australia

<sup>2</sup>USDA/ARS Children's Nutrition Research Center, Department of Pediatrics

<sup>3</sup>Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston TX 77030

Redirection of available energy accompanies dynamic changes in physiological processes. Lactation places the greatest energy demand during the female life-cycle. This requires a sustained increase in maternal metabolism, often resulting in a change in body weight and body composition. Impaired regulatory responses to such a metabolic challenge may lead to lactation failure and have serious consequences for neonatal survival, or at least impact growth and development. We used a complex phenotyping strategy to measure variation across 32 inbred strains of mice from the diversity panel, during lactation. An *in silico* genome-wide association analysis was performed to identify regions that contribute to energy expenditure and body composition during lactation. Dams and their litters were studied for 8 days postpartum during second lactation. On day 1 postpartum (L1), 10 one-day old CD1 pups were cross-fostered to each lactating dam to standardise variation in pup suckling drivers during the study. Food intake and litter weight gain were recorded daily. Data for food intake and milk energy output (litter weight gain) between day 7 (L7) and day 8 postpartum (L8), were used to calculate energy balance. Body composition was measured using quantitative magnetic resonance (QMR) on L1 and again at L8 to assess changes during the study period. Genotyping was based on 132k SNP genotypes from across the inbred strains, and derived haplotag SNPs were then utilized for genome-wide association analysis. Significant strain differences ( $P < 0.001$ ) were observed in food intake, milk energy output, maternal body weight variation, and body composition during lactation. GWAS revealed seven regions associated with change in body weight, five associated with fat mass change, three associated with milk energy output, 32 associated with food energy intake and 1 associated with energy balance. The study demonstrates utility of the mouse diversity panel in analysis of complex traits.

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## Poster Abstracts

### **P-001: Abnormal Innate Immune Responses of ENU-induced *Alii8* and *Alii4* Mutant Mice Lead to Autoinflammatory Syndrome-like Phenotypes**

Koichiro Abe<sup>\*1</sup>, Satochi Nunomura<sup>2</sup>, Chisei Ra<sup>2</sup>, Atsushi Tajima<sup>3</sup>, Helmut Fuchs<sup>4</sup>, and Martin Hrabe de Angelis<sup>4</sup>

<sup>1</sup>Tokai University

<sup>2</sup>Nippon University

<sup>3</sup>Kanazawa University

<sup>4</sup>Helmholtz Zentrum Muenchen

Autoinflammatory syndromes are associated with acute spontaneous inflammation such as painful abdomen and extremities. Unlike autoimmune diseases, autoinflammatory syndromes are caused by defects in the innate immunity. However, the molecular mechanism underlying pathogenesis of autoinflammatory syndromes is poorly understood. In a mouse ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis screen, *Alii8* and *Alii4* dominant mutant strains were established because of spontaneous inflammation on peripheral paws. By genetic mapping and candidate sequencing, *Alii8* and *Alii4* are identified as gain-of-function mutations in a protein kinase and phospholipase C gamma2, respectively. Bone marrow transfer and other immunological experiments indicate that innate immune cells contribute to trigger autoinflammation in both *Alii8* and *Alii4* mutant mice. We assumed that mast cells are possible candidates to initiate autoinflammation in mutants, because they are bone marrow-derived, settle in various tissues, such like mucosal membrane and epidermis, and release various inflammatory mediators. To know contribution of mast cells to the inflammatory phenotypes, *Alii8* and *Alii4* mutant mice were crossed with *W* mutant mice as *W/W<sup>v</sup>* alleles have almost no mast cells in tissues. Interestingly, double mutants with *Alii8* and *W/W<sup>v</sup>* (*Alii8*; *W/W<sup>v</sup>*) showed no autoinflammation in peripheral paws, but *Alii4*; *W/W<sup>v</sup>* alleles showed inflammation on paws. In addition, we analyzed activity of cultured mast cells derived from bone marrow of *Alii8* and *Alii4* mice. Although abnormal degranulation was not detected in mast cells from mutant mice, non-canonical mobilization of cytoplasmic calcium concentration was detected when stimulated by IgE cross-linking. The gene expression patterns of cytokines and chemokines in mast cell from mutant mice are under analysis. These results strongly suggest that mast cells are contribute to initiate autoinflammation in *Alii8* mutant mice. In *Alii4* mutant mice, however, partly mast cells but other myeloid cells and monocytes could be more important to trigger spontaneous inflammation.

**P-002: *Xist/Tsix* expression dynamics during mouse peri-implantation development revealed by whole-mount 3D RNA-FISH**

Hirosuke Shiura, and Kuniya Abe\*

Technology & Development Team for Mammalian Genome Dynamics, RIKEN BioResource Center, Ibaraki 305-0074, Japan

During peri-implantation development in mice, the X Chromosome inactivation (XCI) status changes dynamically. However, the occurrence of these processes *in vivo* has not been explored in detail. To delineate the changes in XCI status *in vivo*, the expression of *Xist* and its antisense partner, *Tsix*, was examined via whole-mount RNA-FISH using strand-specific probes. In the embryonic cell lineage, *Xist* RNA began to disappear at embryonic day 3.75 (E3.75) and was lost completely by E4.5, whereas the derepression of *Tsix* from the silenced allele of the inactive X lagged behind the erasure of *Xist*. *Tsix* biallelic expression became dominant at E5.0, while the *Xist* cloud was visible again in some nuclei of E5.25 epiblasts. Considering that *Tsix* biallelic expression is a sign of epigenetic equivalency of two X Chromosomes, imprinted XCI reversal is likely completed by E5.0, and random XCI starts immediately after the completion of imprinted XCI erasure. Moreover, these results suggest that *Tsix* expression is dispensable for *Xist* downregulation during the erasure process. Intriguingly, epiblast cells exhibiting biallelic expression of *Xist* including two *Xist* clouds per nucleus were observed frequently (~15%) at E5.25 and E5.5. Although imprinted XCI appeared to be stable in the primitive endoderm-visceral endoderm lineage, transient loss of *Xist* clouds was noted in a subset of extraembryonic ectodermal cells, suggesting distinct features of XCI among the three different embryonic tissue layers. These results will serve as a basis for future functional studies of XCI regulation *in vivo*.

### **P-003: Towards the Creation of Reference Transcription Start Site Set (refTSS)**

Imad Abugessaisa<sup>\*1</sup>, Shuhei Noguchi<sup>1</sup>, Akira Hasegawa<sup>2</sup>, Hideya Kawai<sup>3</sup>, and Takeya Kasukawa<sup>1</sup>

<sup>1</sup>RIKEN Center for Life Science Technologies Division of Genomic Technologies Life Science Accelerator Technology Group Large Scale Data Managing Unit

<sup>2</sup>RIKEN Center for Life Science Technologies Division of Genomic Technologies Life Science Accelerator Technology Group Genomics Algorithms Development Unit RIKEN Center for Life Science Technologies Center Director's Strategic Program Molecular Network Control Research Project Molecular Network Control Genomics Unit

<sup>3</sup>RIKEN Research Cluster for Innovation RIKEN Preventive Medicine and Diagnosis Innovation Program RIKEN Center for Life Science Technologies Division of Genomic Technologies Life Science Accelerator Technology Group Genome Information Analysis Team

Reference data sets in genomics, such as reference genomes and reference genes, are of great importance to enable researchers to access integrated and annotated data with specific focus and scope, and to compare their own data against other data on the reference data set.

Several international efforts have been dedicated to generate transcription start site (TSS) and promoter data using sequence technologies such as CAGE. Despite the vast number of TSS data sets available, we still lack integrated reference TSSs.

Therefore, the refTSS project aims to construct a comprehensive TSS data set. Through manual and automated procedures, the refTSS will provide an integrated and well-annotated data set based on the published FANTOM5 promoter atlas and the publically available TSS and promoter data set. In particular, the refTSS platform will integrate various data sets based on TSS, such as TSS activity, gene annotation, regulation, epigenetics, and segmentation information.

Our approach to construct refTSS is based on three components. The first is the raw data sets for genomic coordination of TSSs, which consist of the 5' end sequence information. The second is the annotation information, such as functional annotation and transcriptional regulation. Annotation information will be integrated by association of annotation to the individual TSS. The third is the human curation procedures required to confirm the identified TSS and the annotation.

This is an ongoing project. We have established the work flow and started verifying our approach. We applied remapping procedures on individual data sets (FANTOM5, EPD, and DBTSS) to the latest genome assembly. The remapping is followed by quality evaluation and TSS identification and integration.

The refTSS will be publically available via web portals. We envision refTSS as an important tool for enabling integrative analysis with different "players" within the TSS field.

#### **P-004: Somatic variations in healthy skin fibroblasts and their relation to cancer and aging**

Alexej Abyzov<sup>\*1,2</sup>, Livia Tomasini<sup>2</sup>, Bo Zhou<sup>3</sup>, Nikolaos Vasmatazis<sup>1</sup>, Anahita Amiri<sup>2</sup>, Jessica Mariani<sup>2</sup>, Yanhong Wu<sup>1</sup>, Denise Walker<sup>1</sup>, Mariangela Amenduni<sup>2</sup>, Michael Wilson<sup>2</sup>, Mark Gerstein<sup>2</sup>, Julie Cunningham<sup>1</sup>, Jin Jen<sup>1</sup>, Sherman Weissman<sup>2</sup>, Alexander Urban<sup>3</sup>, and Flora Vaccarino<sup>2</sup>

<sup>1</sup>Mayo Clinic, Rochester, MN, 55905

<sup>2</sup>Yale University, New Haven, CT, 06520

<sup>3</sup>Stanford University, Palo Alto, CA, 94305.

Only a few studies have been conducted to understand natural somatic mosaicism, that is post-zygotic accumulation of mutations in cells of multicellular organisms. Fundamental knowledge about somatic mosaicism is not only crucial for finding determinants of cancer development and progression, but also for an understanding of various diseases and aging. For ten people, we have compared genomes of over twenty clonally derived human induced pluripotent stem cell (hiPSC) lines to the genomes of four primary skin fibroblast samples, parental to the hiPSC lines. The clonal nature of hiPSC lines allows the discovery of somatic genomic variants present in the founder cell, but not in all fibroblast cells, thereby providing a mean for a high-resolution analysis of single cell genomes. With this approach we found that, on average, an iPSC line derived from children manifests 400 single nucleotide variants (SNVs) not apparent in the fibroblasts. We next performed an in-depth re-evaluation of these candidate somatic variants in the fibroblasts with three orthogonal experimental techniques. These experiments confirmed that at least 20% (roughly 80 SNVs), and up to 70% (roughly 270 SNVs), of the candidate SNVs are mosaic somatic variants in fibroblast cells. The allele frequency of these SNVs ranged from 0.1% to a dozen percent in the fibroblast cell population, and the mutation spectrum was surprisingly similar to that observed in some cancers (Alexandrov et al., Nature, 2013). Analysis of hiPSC derived from fibroblasts of children's parents indicates that parents have 1.5 to 2 times more mosaic SNVs per cell. These new discoveries emphasize a large degree of somatic mosaicism existing in healthy human tissues, provide the first evidence that mutational signatures observed in cancers could be attributed to a background somatic mosaicism in normal cells, and demonstrate that the amount of somatic mosaicism increases with age.

## **P-005: Elevated canonical Wnt signalling disrupts development of the embryonic midline and can cause Heterotaxy**

Alaa Alzahrani, Koula Diamand, Jehangir Ahmed, Kristen Barratt, and Ruth Arkell\*

Early Mammalian Development Laboratory, Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia

Heterotaxy is a congenital abnormality where the internal thoraco-abdominal organs demonstrate abnormal arrangement across the left-right (L-R) axis of the body. It can affect the development of the heart, liver, lungs, intestines, and spleen. The L-R embryonic axis is established early in embryogenesis when unidirectional signals emanate from a specialised structure at the embryonic midline, called the node, to initiate distinct molecular pathways on the left and right sides of the developing embryo. The gene most commonly mutated in human cases of Heterotaxy is the X-linked *ZIC3*, but the mechanism by which the *ZIC3* transcription factor prevents Heterotaxy remains unknown. A genetic screen for mutations that affect murine embryogenesis identified *katun (Ka)*, a novel null allele of *Zic3*. The mutant embryos exhibit Heterotaxy and also incompletely penetrant, partial (posterior) axis duplications and anterior truncation. These latter two phenotypes are redolent of elevated canonical Wnt signalling and analysis of *Ka* embryos reveals ectopic expression of direct targets of Wnt/ $\beta$ -catenin mediated transcription in mutant embryos. *ZIC3* is a member of the *Zic* family of transcriptional regulators and previous work has shown that *ZIC* proteins can inhibit Wnt/ $\beta$ -catenin mediated transcription when overexpressed in cell lines. This raises the possibility that dysregulated Wnt signalling may contribute to Heterotaxy. We have investigated this notion by analysis of the murine batface (*Bfc*) gain-of-function allele of  $\beta$ -catenin that results in elevated Wnt/ $\beta$ -catenin signalling. We find this strain exhibits incompletely penetrant defects of L-R axis formation, caused by a defective midline development, and synergises with the *Zic3 Ka* allele to produce an increased incidence of L-R axis defects. Moreover we find that human *ZIC3*-Heterotaxy associated mutations encode proteins that are defective in their ability to inhibit Wnt/ $\beta$ -catenin mediated transcription. Overall this provides strong evidence that Wnt dysregulation can contribute to human cases of Heterotaxy.

## **P-006: Targeted reduction of highly abundant transcript with pseudo-random primers**

Ophelie Arnaud\*, Stephane Poulain, Sachi Kato, and Charles Plessy

RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Yokohama, Kanagawa, 230-0045 Japan.

Since a few years, several methods to study the single cell transcriptome have been developed. Most of them use poly-T oligonucleotides in the reverse transcription step, which limits the detection of non-poly(A) transcripts. However, given the importance of the non-coding RNA, random-priming the RNA should give a more complete transcriptome. Unfortunately, the use of random primers produces a high proportion of reads coming from ribosomal RNA, decreasing the depth of the sequencing. Moreover, because of the sequencing cost, the single cells are multiplexed, which limits the total number of sequenced read per single cell. As a consequence, only the most abundant transcripts in a cell are detected. Therefore, the depletion of the abundant and undesirable transcripts, like the ribosomal RNA one, before the sequencing will improve the detection of the lower expressed transcripts. However, as the RNA starting quantity is very low, an extra step (like Ribo-Zero) isn't possible. Thus, we propose to use a selective set of random primers, the pseudo-random (PS) primers, allowing the detection of the non-poly(A) RNA while also limiting the detection of the ribosomal RNA. Here, we are presenting, the proof of concept of the PS primers in the nanoCAGE protocol, starting with 50ng of total RNA.

We have demonstrated that the use of only 40 PS primers instead of the 4096 random primers considerably decreased the reads coming from ribosomal RNA sequences without affected the transcriptome diversity or the number of genes detected. We have also demonstrated that this strategy can be applied to deplete a sample of other undesirable sequences like the sequences coming from the hemoglobin genes.

In conclusion, the pseudo-random primers are effective for eliminating specific and unrelated sequences without affecting the gene coverage. They are simple to design, and therefore are flexible tool for depleting any transcriptome libraries in abundant and undesirable sequences.

## **P-007: F5-explorer: an interactive webserver for quick gene-oriented browsing of FANTOM5 data**

Frederik Otzen Bagger<sup>\*1,2,3,4,7</sup>, Sina Hadi Sohi<sup>5</sup>, Mette Louise Trempenau<sup>1,2,4</sup>, Maria Dalby<sup>3</sup>, Damir Sasivarevic<sup>5</sup>,  
Bo Torben Porse<sup>1,2,4</sup>, Ole Winther<sup>5</sup>, The FANTOM consortium, Robin Andersson<sup>3</sup>, and Nicolas Rapin<sup>1,2,3,4</sup>

<sup>1</sup>The Finsen Laboratory, Rigshospitalet, Faculty of Health Sciences, University of Copenhagen, Denmark

<sup>2</sup>Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Copenhagen, Denmark

<sup>3</sup>The Bioinformatics Centre, Department of Biology, Faculty of Natural Sciences, University of Copenhagen

<sup>4</sup>Danish Stem Cell Centre (DanStem) Faculty of Health Sciences, University of Copenhagen, Denmark

<sup>5</sup>DTU Compute, Technical University of Denmark, Lyngby, Denmark

<sup>6</sup>The FANTOM Consortium

<sup>7</sup>current address: European Molecular Biology Laboratory - European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus, Hinxton, Cambridge, UK, and Department of Haematology, University of Cambridge, Cambridge Biomedical Campus, Cambridge CB2 0PT, UK

A large and comprehensive body of data has been laid out by the FANTOM5 project covering transcription start site usages across a wide range of cell and tissue types. Here, we present a gene-oriented interface for accessing and visualizing the data via a web-browser: the F5-explorer.

The F5-explorer offers a simple interface to plot expression levels of promoters or enhancers associated with a single queried gene. The platform utilizes annotations of samples to structured ontologies (Cell Ontology, Uberon, Disease Ontology) provided by the FANTOM consortium and a simplified tree-like hierarchical representation provided directly on the interface makes it possible quickly to browse different cell types or tissues of the human body. The interface does not encompass the full body of data, nor displays all details of the metadata and ontology annotations, but rather attempts to simplify and speed up the process of answering simple, yet important, biological questions for biologists with or without bioinformatics skills. For predetermined users, or users in need of a specific plot or comparison not available from the general default terms a shopping basket functionality has been built into the F5-explorer. This allows for creating any user-defined plot or comparison from the data and furthermore expands functionality of the interface to include the entirety of the FANTOM5 data.

In conclusion, we present a new gene-oriented interface for quick browsing of the FANTOM5 data. By metadata pruning and an interactive graphical user-interface, we present a portal to one of the most comprehensive and encompassing transcriptomics datasets available to date. We believe that this platform will further increase the availability and use of the FANTOM5 consortium effort and broaden its potential users.

The web interface is freely available and requires no login at: <http://servers.binf.ku.dk/fantom5>



## P-008: Multimer formation explains allelic suppression of PRDM9 recombination hotspots

Christopher Baker<sup>\*1</sup>, Pavlina Petkova<sup>1</sup>, Michael Walker<sup>1</sup>, Petr Flachs<sup>2</sup>, Ondrej Mihola<sup>2</sup>, Zdenek Trachtulec<sup>2</sup>, Petko M Petkov<sup>1</sup>, and Kenneth Paigen<sup>1</sup>

<sup>1</sup>Center for Genome Dynamics, The Jackson Laboratory, Bar Harbor, ME 04609, USA

<sup>2</sup>Laboratory of Germ Cell Development, Division BIOCEV, Institute of Molecular Genetics of the Academy of Sciences of the Czech Republic, v. v. i., Videnska 1083, CZ14220 Prague, Czech Republic

Genetic recombination during meiosis functions to increase genetic diversity, promotes elimination of deleterious alleles, and helps assure proper segregation of chromatids. Mammalian recombination events are concentrated at specialized sites, termed hotspots, whose locations are determined by PRDM9, a zinc finger DNA-binding histone methyltransferase. *Prdm9* is highly polymorphic with most alleles activating their own set of hotspots. Most mammalian populations exhibit high frequencies of heterozygosity; however, questions remain about the influences different alleles have in heterozygous individuals where the two variant forms of PRDM9 typically do not activate equivalent populations of hotspots. We now find that, in addition to activating its own hotspots, the presence of one *Prdm9* allele can modify the activity of hotspots activated by the other allele. PRDM9 function is dosage sensitive; B6-*Prdm9*<sup>+</sup>/*Prdm9*<sup>m1Ymat</sup> heterozygous null mice have reduced numbers and less active hotspots and increased numbers of aberrant germ cells. In mice and humans carrying two *Prdm9* alleles, there is allelic competition; the stronger *Prdm9* allele can partially or entirely suppress chromatin modification and recombination at hotspots of the weaker allele. In cell cultures, PRDM9 protein variants form functional heteromeric complexes which can bind hotspot sequences. When a heteromeric complex binds at a hotspot of one PRDM9 variant, the other PRDM9 variant, which would otherwise not bind, can still methylate hotspot nucleosomes. We propose that in heterozygous individuals the underlying molecular mechanism of allelic suppression results from formation of PRDM9 heteromers, where the DNA binding activity of one protein variant dominantly directs recombination initiation towards its own hotspots, effectively titrating down recombination by the other protein variant. In natural populations with many heterozygous individuals, allelic competition will influence the recombination landscape.

## **P-009: Influence of diet on metabolic syndrome in four genetically diverse mouse strains**

William Barrington<sup>\*1,3</sup>, Daniel Pomp<sup>2</sup>, Brian Bennett<sup>2</sup>, and David Threadgill<sup>3</sup>

<sup>1</sup>North Carolina State University and Texas A&M University

<sup>2</sup>University of North Carolina Chapel Hill

<sup>3</sup>Texas A&M University

Recent research has demonstrated the profound impact of altering dietary macronutrient composition on mouse health in C57BL/6J mice. However, there is little research exploring how relevant human diets affect health in genetically diverse mice. Our study addressed this gap in knowledge by assessing metabolic syndrome-related phenotypes in four genetically diverse mouse strains (A/J, C57BL/6J, FVB/NJ, NOD/ShiltJ) fed one of five human diets (Western, Mediterranean, Japanese, Hunter-Gatherer, and Ketogenic) or a standard mouse chow. Adiposity, blood pressure, liver triglycerides, blood parameters, and mitochondrial function were assessed. Each strain had substantially different reactions to the diets. A/J mice were generally resistant to negative impacts from all diets. C57BL/6J performed poorly on the high-fat Western diet, but had positive effects when fed a high-fat ketogenic diet. Oppositely, the ketogenic diet produced strong, negative effects on metabolic syndrome phenotypes in FVB/NJ mice that did better on a Western diet. Detailed effects on background and diet-dependent development of metabolic syndrome will be presented. The data indicates that there is profound individual variation in diet response that is not detectable in human epidemiological studies. Follow-up studies are underway to determine the genetic causes underlying these diverse diet responses.

**P-010: Characterization and mapping of *colimba*, a new spontaneous mouse mutation with hair coat abnormalities.**

David Bazaga<sup>1</sup>, Carlos Perez<sup>1,2</sup>, Joe Angel, and Fernando Benavides<sup>\*1</sup>

<sup>1</sup>Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Smithville, Texas, USA

<sup>2</sup>Division of Pharmacology & Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, Texas, USA

In this study we present *colimba* (*col*), a new autosomal recessive mutation with early growth retardation and sparse hair coat. The mutation appeared in a C57BL/6.Ps1E<sup>dba</sup> congenic strain and was maintained as a homozygous line. The initial mapping using (C57BL/6 x FVB/N)F2-*col/col* mice allowed us to localize the *col* locus to proximal Chromosome 1, between the centromere and marker *D1Mit373* (26.4 Mb), a region of homology with human Chromosomes 8q11-13 and 6q12. The phenotype of *col/col* mice is obvious a few days after birth and includes retardation in post-natal development and partial alopecia. Histologically, the skin shows altered hair follicle cycle at several time points. No other histological lesions were observed. Furthermore, tumor multiplicity after chemical skin carcinogenesis is higher in mutant mice than wild-type littermates. There are no obvious functional candidate genes for the *col* mutation in the candidate region; however, we will present the analysis of complete exome sequencing.

## **P-011: Cilia and Ciliopathies: Impact of new knowledge on our understanding of biology and human disease**

Karen Christie, Janan Eppig, Cynthia Smith, Hiroaki Onada, Joel Richardson, and Judith Blake \*

The Jackson Laboratory, Bar Harbor, ME USA

Interest in primary cilia has increased dramatically as it has become clear that ciliopathies are an underlying cause of numerous human diseases including for some types of retinitis pigmentosa , for polycystic kidney disease, and for cardiovascular pathologies. Once thought to be restricted to a few cell types, it is now apparent that primary cilia are found on almost all vertebrate cells and are critical to sonic hedgehog (*Shh*) signaling. Mouse models play a key role in developing our understanding of the role of cilia in control of *Shh* signaling in development throughout the embryo, and in ongoing maintenance of structures such as photoreceptors. □ □

To maximize the utility of the wealth of experimental data generated by mouse ciliopathy models, we have engaged in a project to comprehensively annotate experimentally characterized ciliary genes of mouse using Gene Ontology (GO) terms to describe their molecular functions, biological roles, and cellular locations, using the SYSCILIA gold standard of known human ciliary components as a starting point. We are updating the GO to add new terms that represent recent advances in our understanding of ciliary biology. Comprehensive GO annotation of ciliary genes in the mouse will be a great resource for researchers engaged in high throughput studies or comparative genomic analysis across species. In addition, Mouse Genome Informatics (MGI) has tools, including the Human-Mouse: Disease Connection interface and MouseMine, to help researchers identify connections between mouse genes and human genes, and to identify relevant mouse models which may be useful in the study of a given disease. We are actively collaborating in the Cardiovascular Development Consortium (CvDC), Bench-to-Bassinet (B2B) program of the National Heart Lung and Blood Institute (NHLBI), and some of the results of that work relative to cilia processes will be presented. □ □

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## **P-012: Single-cell transcriptomes of fluorescent, ubiquitination-based cell cycle indicator cells**

Michael Boettcher<sup>\*1</sup>, Tsukasa Kouno<sup>1</sup>, Elo Madisson<sup>2</sup>, Efthymios Motakis<sup>1</sup>, Imad Abugessaisa<sup>1</sup>, Sachi Kato<sup>1</sup>, Harukazu Suzuki<sup>1</sup>, Yoshihide Hayashizaki<sup>3</sup>, Takeya Kasukawa<sup>1</sup>, Piero Carninci<sup>1</sup>, Timo Lassmann<sup>4</sup>, Jay W Shin<sup>1</sup>, and Charles Plessy<sup>1</sup>

<sup>1</sup>RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Yokohama, Kanagawa, 230-0045 Japan

<sup>2</sup>Karolinska Institutet, Department of Biosciences and Nutrition, SE-141 83 Huddinge, Sweden

<sup>3</sup>RIKEN Preventive Medicine & Diagnosis Innovation Program, Yokohama, Kanagawa, 230-0045 Japan

<sup>4</sup>Telethon Kids Institute, West Perth Western Australia 6872, Australia

Using HeLa cells that report their cell cycle phase through fluorescent, ubiquitination-based cell cycle indicators (*Fucci*), we produced a reference dataset of more than 270 curated single cells for which each single-cell's transcriptome can be matched with cell cycle information via the fluorescence intensity of the transgenes in that cell. We developed a comprehensive open data management and quality control pipeline that enables users of our dataset to process all available sequence and image files in a highly reproducible way without prior knowledge of the underlying bioinformatical toolset. The final output of that pipeline is a customizable table with relevant metadata and quality information for each single cell. This metadata table can be easily used as input for sophisticated data analysis. Our workflow is also adjustable for usage with other single-cell datasets that consist of RNA-sequencing and fluorescence data. Currently, we use the *Fucci* dataset to create a model for cell cycle phase inferences, which can be applied to other single-cell transcriptomes without cell cycle phase reporting metadata.

**P-013: Analysis of ENU mutant mice indicates the existence of non-exomic thrombosis modifier mutations**

Marisa A Brake<sup>\*1</sup>, Amy E Siebert<sup>1</sup>, Kart Tomberg<sup>2</sup>, Guojing Zhu<sup>2</sup>, David R Siemieniak<sup>2</sup>, and Randal J Westrick<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Oakland University, Rochester, Michigan, USA

<sup>2</sup>Life Sciences Institute, University of Michigan, Ann Arbor, Michigan, USA

A variant of coagulation Factor V called Factor V Leiden (FVL) is a potent, yet incompletely penetrant, risk factor for venous thromboembolism (VTE). We sought to understand the genetic basis for this incomplete penetrance by using a FVL ( $F5^{tm2Dgi}$ ) mouse model. We identified a perinatal lethal phenotype in homozygous FVL mice also heterozygous deficient for tissue factor pathway inhibitor ( $F5^{tm2Dgi}/F5^{tm2Dgi} Tjpi^{tm1Gjb/+}$ ). This life/death phenotype formed the basis for a binary sensitized whole genome ENU mutagenesis screen for dominant thrombosis suppressors. ENU mutagenized  $F5^{tm2Dgi}/F5^{tm2Dgi} Tjpi^{tm1Gjb/+}$  males were bred to  $F5^{tm2Dgi}/F5^{tm2Dgi}$  females and surviving  $F5^{tm2Dgi}/F5^{tm2Dgi} Tjpi^{tm1Gjb/+}$  offspring inherited an ENU induced mutation that suppresses the lethal phenotype. Screening of 6,754 progeny identified 98 mice with putative suppressors. Of these, 16 were successfully mated back to  $F5^{tm2Dgi}/F5^{tm2Dgi}$  to create thrombosis suppressor lines. Three of these lines, *MF5L5*, *MF5L14*, and *MF5L22* exhibited penetrances of 58.9%, 33.9%, and 80.8%, respectively. Five mice from each of these lines were whole exome sequenced to identify candidate suppressor mutations. *MF5L5* had 6 candidates, *MF5L14* had 5 candidates, and *MF5L22* had 22 candidates. To determine the actual suppressor mutation among the candidates, a minimum of 12 mice from multigenerational pedigrees were genotyped for the presence of the mutations. Kaplan-Meier survival curves for each putative modifier were insignificant. In addition,  $F5^{tm2Dgi}/F5^{tm2Dgi} Tjpi^{tm1Gjb/+}$  mice with none of the original exomic candidates were observed in succeeding generations. Thus, whole genome sequencing is the best option for identifying the suppressor mutants in these three lines and sequencing of mice from each line is in progress. The identification of thrombosuppressive modifiers will provide novel insights into the pathways leading to VTE and facilitate novel therapeutic interventions.

## **P-014: Shape-based morphometric analysis of homozygous lethal embryos imaged by micro-CT**

James M Brown<sup>\*</sup>, Neil Horner, James Cleak, Sara Johnson, Zsombor Szoke-Kovacs, Lydia Teboul, Carl Henrik Westerberg, and Ann-Marie Mallon

Mammalian Genetics Unit, MRC Harwell, Harwell Science and Innovation Campus, Harwell OX11 0RD, UK

The International Mouse Phenotyping Consortium (IMPC) (<https://www.mousephenotype.org>) is a multi-national effort to functionally characterize each of the 20,000 genes in the mouse genome. Of the 2,400 knockout lines assessed so far, approximately 30% are embryonic or perinatal lethal. In order to establish the cause of lethality, 3D imaging techniques such as micro-CT, OPT and HREM are employed at several key developmental stages to capture aberrant embryo morphology.

The high-throughput nature of the screening process makes manual annotation of 3D imaging data infeasible, and subtle phenotypes may be overlooked by human experts. Automated methods for phenotype detection in 3D images often rely on image registration, where wild-type and mutant embryos are brought into spatial alignment with one another. Voxel or deformation-based morphometry is then used to elucidate statistically significant differences between the two groups. With the addition of an appropriately labelled atlas, differences in total organ volume may also be measured. Such an approach is highly amenable to further analysis, based on shape-based (surface) representations of organs.

Statistical shape models (SSMs) are used to extract the geometric variation from a set of input objects. Using such a model, individual shapes (such as organs) may be represented as deviations from the average shape, allowing for morphological differences to be readily identified. In this work, we propose a novel method for constructing multi-organ SSMs from micro-CT images of E14.5 embryos, having been registered towards an average atlas. The proposed method was applied to a number of knockout mouse lines with known phenotypes, and the results compared to those produced using existing methods. We demonstrate that an SSM-based approach provides a means of assessing embryo organ morphology that is complementary to existing methods, as well as providing additional information about abnormal organ morphology.

**P-015: The JAX Synteny Browser: A new visualization tool for mouse-human comparative genomics**

Paul Hale, Keith Sheppard, Mei Xiao, Joel Richardson, and Carol J Bult\*

The Jackson Laboratory, Bar Harbor, Maine, 04609

We have developed a new web-based synteny browser to support visualization of conserved synteny between the mouse and human reference genomes. In contrast to existing synteny visualization tools, the JAX Synteny Browser is tightly integrated with the rich biological annotations of mouse genes available from the Mouse Genome Informatics (MGI) database. The browser allows users to navigate to specific regions of conserved synteny and then to highlight or filter the features displayed according to specific biological properties including gene function, phenotype, or disease. The browser supports four distinct levels for visualization: genome wide, chromosome, block, and feature level. All of the underlying data needed for the browser are expected to conform to common bioinformatics data format standards. For example, genome features are represented in GFF3; functional annotations are represented in a GAF format; variants are represented in VCF files, etc. The software has been developed in alignment with the philosophy behind other "generic model organism database (GMOD)" software products so that the visualization tool can be extended easily to other pairs of organisms.

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## P-016: Modeling Binding Affinity of the Multiple Zinc-Finger Protein PRDM9

Alexander Fine<sup>1,2</sup>, Michael Walker<sup>1</sup>, Timothy Billings<sup>1</sup>, Kenneth Paigen<sup>1</sup>, Petko M Petkov<sup>1</sup>, and Gregory W Carter<sup>\*1,2</sup>

<sup>1</sup>The Jackson Laboratory, Bar Harbor, ME 04609

<sup>2</sup>Tufts University School of Medicine, Boston, MA 02111

Mammalian genomes encode hundreds of zinc finger proteins (ZFPs) that bind DNA, but most have unknown functions. One well-characterized example, PRDM9, recognizes and binds with multiple tandem zinc fingers to regulate genomic locations of meiotic recombination. Over 20 alleles of PRDM9 are known in mice, each containing a unique array of zinc fingers. Each allele is expected to bind a different set of loci, providing a natural experimental system for investigating how ZFPs recognize DNA sequence. To study site selection, we used a novel, *in vitro* sequencing strategy called Affinity-Seq to assess binding of PRDM9 to genomic DNA. We found over 30,000 significant binding sites for the PRDM9<sup>Dom2</sup> isoform and quantified the frequency of binding at each sequence. This enabled estimation of binding affinity at each site in addition to standard nucleotide frequencies. The vast majority (95%) of sites contained an allele-specific binding motif, suggesting a single binding sequence for each PRDM9 isoform. We identified a few core nucleotides required for binding. However, analysis of F1 hybrid mice suggested variability at all bases affects binding frequency. To assess the importance of each nucleotide, we performed linear regression to model effects on binding affinity. Quantitative data for thousands of sites allowed us to infer additive and interactive effects for all bases, revealing multiple nucleotide-nucleotide interactions that drive binding. We tested this model by performing Affinity-Seq on a second genome, providing thousands of natural polymorphisms for quantitative validation. Our work provides a detailed view of DNA binding by PRDM9 and unprecedented power to assess the complex rules of zinc finger binding specificity. Models based on these rules can potentially predict novel recombination hotspots and the functions of uncharacterized ZFPs.

**P-017: Mapping Genomic Distributions of Combinatorial Histone Modifications at the Single Molecule Level**

Jen-Chien Chang<sup>\*1</sup>, Takashi Umehara<sup>2</sup>, Keisuke Fujita<sup>3</sup>, Yuichi Taniguchi<sup>4</sup>, Toshio Yanagida<sup>3</sup>, and Akiko Minoda<sup>1</sup>

<sup>1</sup>Division of Genomic Technologies, RIKEN Center for Life Science Technologies

<sup>2</sup>Division of Structural and Synthetic Biology, RIKEN Center for Life Science Technologies

<sup>3</sup>Laboratory for Cell Dynamics Observation, RIKEN Quantitative Biology Center

<sup>4</sup>Laboratory for Single Cell Gene Dynamics, RIKEN Quantitative Biology Center

Post-translational modifications of histones, which mediate many nuclear processes, is one of the most well characterized epigenetic mechanisms. In recent years, it has become evident that specific combinations of histone modifications represent various chromatin states, and are associated with different regulatory functions. However, standard methods probe one histone modification at a time; thus, the combinatorial patterns at each histone or nucleosome are largely unknown. Here, we develop a novel method for mapping the genomic distribution of combinatorial histone modifications by integration of two platforms: single molecule fluorescent microscope and the next-generation sequencer. Once this method is established, we will first investigate the genomic localization of coexisting active and repressive histone marks in different cell types. Other applications include the colocalization of histone variants, DNA methylation, transcription factors, and chromatin-binding proteins. By combining other data sets such as RNA-seq, transcription factors ChIP-seq, and DNase Hypersensitivity assay, we aim to identify the functions of chromatin states, with the ultimate goal in providing a complete picture of how epigenome is modulated.

**P-018: Zebrafish serves as a disease model system to investigate the embryonic development effects of human parvovirus B19- NS1 and VP1u protein**

Ju Chang-Chien<sup>\*1,2</sup>, Jiann-Jou Yang<sup>2</sup>, Tsai-Ching Hsue<sup>1</sup>, and Wei-Jen Chen<sup>2</sup>

<sup>1</sup>Institute of Biochemistry, Microbiology and Immunology, College of Medicine, Chung Shan Medical University, Taichung, Taiwan

<sup>2</sup>Department of Biomedical Sciences, Chung Shan Medical University, Taichung, Taiwan

Parvovirus B19 (B19) is known as a human pathogen and has been associated with a variety of disorders including fetal anemia, cardiomegaly, pericardial effusion, hydropic or nonhydropic intrauterine fetal death (IUFD) and autoimmune diseases. To investigate the influences of B19 on embryo development, zebrafish animal model was adopted to elucidate the effects of B19-NS1 and B19-VP1u on embryonic development of zebrafish. Abnormal embryos development from 6 hpf to 96 hpf were observed after injected with B19-NS1 or VP1u expression plasmid. The abnormal phenotype revealed a progressive and defective rate in B19-NS1 or VP1u injected embryos as compared to the controls. In addition, embryos injected with B19-NS1 or B19-VP1u showed the heart edema or abnormal somite development at 96 hpf. However, higher mortality rate was detected in B19-VP1u injected embryos than those embryos injected with B19-NS1. In this study, our data suggests that both B19-VP1u and B19-NS1 proteins interfere the embryonic development and could provide a clue in understanding the possible pathogenic mechanism of B19 in embryonic development.

## **P-019: Investigation of defective spermiogenesis in a human XLID mouse model**

Chun-Yu Chen<sup>\*</sup>, Chih-Hsiang Yu, I-Shing Yu, Yu-Chen Hsu, Ming-Shian Tsai, Meng-Ni Fan, and Shu-Wha Lin

Department of Clinical Laboratory Sciences and Medical Biotechnology, College of Medicine, National Taiwan University, Taipei, Taiwan

Generation of mature spermatozoa requires timely activation and removal of regulators to accommodate the goals in each distinct stage during spermatogenesis. Although it is known that ubiquitination plays important roles in the progression of spermatogenesis, it is unclear whether a key protein degradation regulator. Ubiquitination of target proteins occurs in the progression of germ cell development; however, key participants that maintain functional spermatogenesis remain largely undefined. We report a human X-linked intellectual disability (XLID)-associated cullin-ring-ligase plays crucial roles in post-meiotic sperm development. We found that this XLID-associated gene is consistently expressed in spermatogonia and dynamically expressed in post-meiotic spermatids but not expressed in meiotic spermatocytes. Mutant male mice are sterile and display a progressive loss of germ cells during spermiogenesis leading to oligoasthenospermia. Epididymides of mutant mice contained very low number of mature spermatozoa with pronounced morphological abnormalities. Although mitosis of spermatogonia and meiosis of spermatocytes appeared unaffected in mutant mice, a significant elevation of apoptosis is presented in spermatids during spermiogenesis from acrosome phase to cap phase. Ultrastructural pathological study confirmed that spermatids develop aberrant acrosome and nuclear morphology in the mutant mice. In conclusion, our findings reveal a critical role for this XLID-associated gene as an acrosomal formation and nuclear condensation regulator in haploid male germ cell differentiation and loss of this it in mice leads to male fertility.

**P-020: MECOM (EVI1 or PRDM3) maintains neuronal stem cell self-renewal through chromatin control over RBPJ recruitment.**

Elaine KY Chung<sup>\*</sup>, Tobias Hohenauer, Fatma Urun, Chee Wee Tee, and Adrian W Moore

Lab for Genetic Control of Neuronal Architecture, RIKEN Brain Science Institute, Japan.

Stem cells divide to either self-renew or create precursor cells destined to differentiate. Failure to balance between these outcomes in the nervous system causes neurological disorders and cancer. NOTCH2 activation drives neuronal stem cell self-renewal by repressing proneural gene expression and altering the cell cycle. However, how pleiotropic NOTCH2 initiates this self-renewal program is unclear. Here we show that MECOM oncogenic locus (encoding EVI1, also called PRDM3), commonly overexpressed in leukemia, acts with NOTCH2 in olfactory neuronal stem cells to repress proneural gene expression and promote the G1/S transition. Upon activation, NOTCH2 utilizes the transcription factor RBPJ to activate transcription, and the target *Hes1* is the core of the neuronal stem cell self-replication regulatory network. EVI1 binds but does not induce the *Hes1* locus. Instead, we find that EVI1 controls histone methylation status and nucleosome positioning at the *Hes1* locus. These changes in chromatin structure create a locus with high potential for RBPJ access, and upon NOTCH2 activation they amplify RBPJ binding and *Hes1* induction. Our data define a chromatin control system that primes stem cells to respond to Notch through enabling activation of the self-renewal program.

**P-021: -----The ORFeome Collaboration: A community resource for expression of most human protein-coding genes**

The ORFeome Collaboration\*

ORFeome Collaboration is an international consortium

We present the ORFeome Collaboration (OC) and the community resources this effort has generated: a genome-wide collection of high-quality, fully sequenced human open-reading-frame (ORF) cDNA clones for studies on human protein structure and function; a searchable, annotated database to locate available clones; and an international distribution network to provide the resource to the research community. The OC clone collection comprises one or more ORF clones for 73% of 20,906 RefSeq human genes and 79% of the 19,022 highly curated Consensus Coding DNA Sequence Project (CCDS) human genes. All clones are provided in a Gateway™ entry vector format for easy transfer of the ORF onto a large number of expression vectors covering most expression systems. The ORFeome Collaboration is out of today the most comprehensive collection of human ORF clones made available to the entire international research community to promote the functional annotation of protein coding genes and their gen products.

## P-022: *KCNQ1* and *CFTR* act as tumor suppressors in colorectal cancer

BLN Than<sup>1,11</sup>, JF Linnekamp<sup>2</sup>, JACM Goos<sup>3</sup>, SH den Uil<sup>3</sup>, RJA Fijneman<sup>3,4</sup>, MG O'Sullivan<sup>12</sup>, DA Largaespada<sup>5,9,10</sup>, A Schumann<sup>1</sup>, GA Meijer<sup>3,4</sup>, Y Zhang<sup>7</sup>, C Hodges<sup>8</sup>, TK Starr<sup>5,6,10</sup>, E Van Den Broek<sup>3</sup>, JP Medema<sup>2</sup>, PM Scott<sup>1</sup>, L Vermeulen<sup>2</sup>, and RT Cormier<sup>\*1,10</sup>

<sup>1</sup>Department of Biomedical Sciences, University of Minnesota Medical School, Duluth, MN, USA

<sup>2</sup>Laboratory of Experimental Oncology and Radiobiology, Academic Medical Center, Amsterdam, The Netherlands

<sup>3</sup>Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands

<sup>4</sup>Netherlands Cancer Institute, Amsterdam, The Netherlands

<sup>5</sup>Department of Genetics, Cell Biology and Development, University of Minnesota Medical School, Minneapolis, MN, USA

<sup>6</sup>Department of Obstetrics, Gynecology and Women's Health, University of Minnesota Medical School, Minneapolis, MN, USA

<sup>7</sup>University of Minnesota Supercomputing Institute, Minneapolis, MN, USA

<sup>8</sup>Department of Pediatrics, Case Western Reserve University, Cleveland, OH, USA

<sup>9</sup>Department of Pediatrics, University of Minnesota Medical School, Minneapolis, MN, USA

<sup>10</sup>University of Minnesota Masonic Cancer Center, Minneapolis, MN, USA

<sup>11</sup>Department of Gastroenterology, Weill Cornell Medical College, New York, NY, USA

<sup>12</sup>College of Veterinary Medicine, University of Minnesota, St. Paul, MN, USA

A role for the ion channel genes *Kcnq1* and *Cftr* in colorectal cancer (CRC) was first indicated by their identification as candidate driver genes in Sleeping Beauty transposon-mediated forward genetic screens in mice. Follow-up mouse genetic studies conducted in the C57BL/6J-*Apc*<sup>Min</sup> background showed that *Kcnq1* deficiency significantly enhanced *Apc*<sup>Min</sup> tumor multiplicity, with some adenomas progressing to adenocarcinoma. *Apc*<sup>Min</sup> *Cftr*(*Cftr*<sup>tm1Cwr</sup>/*Cftr*<sup>tm1Cwr</sup>)-deficient mice also showed significant increase in tumor multiplicity and progression and loss of *Cftr* alone was sufficient for development of tumors in >60% of *Apc*<sup>+/+</sup> *Cftr*<sup>tm1Cwr</sup>/*Cftr*<sup>tm1Cwr</sup> (*Cftr* KO) mice.

Further, we found that maintenance of *KCNQ1* protein expression was associated with improved survival in patients undergoing resection for Stage IV colorectal cancer liver metastases with a significant increase in median overall survival of 23 months. This finding was confirmed by enhanced disease-free survival (DFS) in Stage II and Stage III CRC patients. A similar enhancement of DFS was observed in early stage CRC patients who maintained *CFTR* expression.

To investigate the basis of protective effects of *KCNQ1* and *CFTR* in CRC we examined global gene expression in *Kcnq1*(*Kcnq1*<sup>tm1Kpfe</sup>/*Kcnq1*<sup>tm1Kpfe</sup>) and *Cftr*-deficient mouse colon and small intestine. Among top gene clusters were genes involved in innate and adaptive immune responses, ion channels, mucins, cancer cell migration and invasion, stress responses and intestinal stem cell-related genes. In tumors isolated from *Apc*<sup>+/+</sup> *Cftr* KO mice we found upregulation of Wnt/beta-catenin target genes and intestinal stem cell genes, a phenotype similar to that observed in *Apc*<sup>Min</sup> mice. To investigate the roles of *Kcnq1* and *Cftr* in the stem cell compartment we examined intestinal organoid cultures from knockout mice and found that organoid outgrowth and delayed differentiation was enhanced in *Kcnq1*<sup>-/-</sup> and *Cftr*<sup>-/-</sup> colons compared with wild type colons.

Overall, our data identify *KCNQ1* and *CFTR* as CRC tumor suppressor genes that can act as prognostic biomarkers and potential therapeutic targets.

## **P-023: Whole-exome sequencing of ENU-induced mutant mice with BALB/c background**

TA de Souza<sup>\*</sup>, S lenne, MAC Rocha de Carvalho<sup>2</sup>, CF Menck<sup>3</sup>, and SG Massironi<sup>2</sup>

<sup>1</sup>Core Facility for Scientific Research, University of Sao Paulo, Brazil

<sup>2</sup>Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Brazil

<sup>3</sup>Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Brazil

Mutant mice are valuable tools for understanding the mechanisms of basic and complex biological processes. Mutagens, as ENU (N-ethyl-N-nitrosourea), were used to induce random mutations in germinative mouse cells, generating mutant lineages with potentially interesting phenotypes. A key challenge is to identify and associate causal variants in DNA to phenotypes in models. Considering that most of all known ENU-induced causal mutations are found in exons, we sequenced exomes from eight ENU-induced mutant mice with BALB/c background presenting interesting phenotypes. A hybridization-based mouse whole-exome capture system was used to construct libraries for massive parallel sequencing. Reads were mapped to mouse reference genome then an SNP calling pipeline was used to create a raw SNP list for each ENU-induced mutant mice and for inbred strains C57BL/6 and BALB/c used as controls. Here we report the optimization of an SNV filtering strategy for discovery of candidates for ENU-induced mutations in mice. Filtering steps were based upon previously mapped regions, type of inheritance, and uniqueness compared to dbSNP and controls. Using this method, we found strong candidate mutations for all mice sequenced, including potential new models for diseases in humans. Putative causative mutations were evaluated by impact prediction tools and validated by Sanger sequencing. Functional investigations of mutant gene products are still in course. In addition, we are also investigating SNP and small INDELS profiles of our C57BL/6 and BALB/c inbred strains, maintained for decades in our breeding facilities. Taken together, our results will be an important source of information for biomedical researchers and to the field of laboratory mouse genetics.

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## **P-024: A selfish genetic element drives recurrent selective sweeps in the house mouse**

John P Didion<sup>\*1</sup>, Andrew P Morgan<sup>1</sup>, Liran Yadgary<sup>1</sup>, Daniel Pomp<sup>1</sup>, Gary A Churchill<sup>2</sup>, and Fernando Pardo-Manuel de Villena<sup>1</sup>

<sup>1</sup>Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC, US

<sup>2</sup>The Jackson Laboratory, Bar Harbor, ME, US

A selective sweep is the result of strong positive selection rapidly driving newly occurring or standing genetic variants to fixation, and can dramatically alter the pattern and distribution of allelic diversity in a population or species. Population-level sequencing data have enabled discoveries of selective sweeps associated with genes involved in recent adaptations in many species. In contrast, much debate but little empirical evidence addresses whether "selfish" genes are capable of fixation, thereby leaving signatures identical to classical selective sweeps - despite being neutral or deleterious to organismal fitness. Previously, we described the novel *R2d2* locus, a large copy-number variant that causes non-random segregation of mouse Chromosome 2 during female meiosis due to meiotic drive. Here, we show that *R2d2* has driven recurrent selective sweeps while having no discernable effect on fitness. We tested multiple closed breeding populations from 6 outbred backgrounds and found that alleles of *R2d2* with high copy number (*R2d2<sup>HC</sup>*) rapidly increase in frequency, and in most cases become fixed in significantly fewer generations than can be explained by genetic drift. A survey of 15 natural mouse populations in Europe and the United States revealed that *R2d2<sup>HC</sup>* alleles are circulating at intermediate frequencies in the wild; moreover, patterns of local haplotype diversity are consistent with recent positive selection. Our results provide direct evidence of populations actively undergoing selective sweeps driven by a selfish genetic element, and demonstrate that meiotic drive can rapidly alter the genomic landscape in favor of mutations with neutral or even negative effect on overall Darwinian fitness. This has broad implications for evolutionary studies: loci that are implicated as drivers of selective sweeps require independent evidence to determine the type of sweep - hard, soft, or selfish.

## P-025: Implication of truncated CABLES1 in agensis of the corpus callosum

TH Tra Dinh<sup>\*1</sup>, Seiya Mizuno<sup>1</sup>, Hiroyoshi Iseki<sup>1</sup>, Saori Mizuno-Iijima<sup>1</sup>, Jun-Dal Kim<sup>2</sup>, Junji Ishida<sup>2</sup>, Satoshi Kunita<sup>3</sup>, Akiyoshi Fukamizu<sup>2</sup>, Fumihiro Sugiyama<sup>1</sup>, and Ken-ichi Yagami<sup>1</sup>

<sup>1</sup>Laboratory Animal Resource Center, University of Tsukuba, Tsukuba, Japan

<sup>2</sup>Life Science Center, Tsukuba Advanced Research Alliance, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan

<sup>3</sup>Center for Experimental Medicine, Jichi Medical University, Shimotsuke, Japan

Agenesis of the corpus callosum (ACC) is a congenital abnormality of the brain structure. More than 60 genes are involved in the corpus callosum development. However, the molecular mechanisms underlying ACC are not fully understood. Previously, we produced a novel transgenic mouse strain, named as BALB/cAJc1-Tg(Actb-rTA,Actb-tTS)138Utr or TAS, carrying genes of the tetracycline-inducible expression system that are not involved in brain development, and inherited ACC was observed in the brains of all homozygous TAS mice. Although ACC was probably induced by transgene insertion mutation, the causative gene and the molecular mechanism of its pathogenesis remain unclear.

In this study, we first performed interphase three-color fluorescence *in situ* hybridization (FISH) analysis and determined that transgenes were inserted into ~12.0Mb distal to the centromere of Chromosome 18. Gene expression analysis and genomic PCR walking showed that the genomic region containing exon 4 of *Cables1* was deleted by transgene insertion and the other exons of *Cables1* were intact. The mutant allele was designated as *Cables1*<sup>Tg(Actb-rTA,Actb-tTS)138Utr</sup> aka *Cables1*<sup>TAS</sup>. Interestingly, *Cables1*<sup>TAS</sup> mRNA consisted of exons 1-3 of *Cables1* and part of the transgene that encoded a novel truncated CABLES1 protein. Homozygous TAS mice exhibited mRNA expression of *Cables1*<sup>TAS</sup> in the fetal cerebrum, but not that of wild-type *Cables1*.

To investigate whether a dominant negative effect of *Cables1*<sup>TAS</sup> or complete loss of function of CABLES1 gives rise to ACC, we produced *Cables1*-null mutant mice (BALB/c-*Cables1*<sup>tm1Utr</sup>). ACC was not observed in *Cables1*-null mutant mice, suggesting that a dominant negative effect of CABLES1<sup>TAS</sup> impairs callosal formation. Moreover, ACC frequency in *Cables1*<sup>+/+</sup>/*Cables1*<sup>TAS</sup> mice was significantly lower than that in *Cables1*<sup>-/-</sup>/*Cables1*<sup>TAS</sup> mice, indicating that wild-type CABLES1 interfered with the dominant negative effect of *Cables1*<sup>TAS</sup>. This study indicated that truncated CABLES1 causes ACC and wild-type CABLES1 contributes to callosal formation.

**P-o26: Association Analysis of -1997 Polymorphism in Upstream Regulatory Site of *COL1A1* with Low Bone Mineral Density: A Study on Iranian Post-menopausal Women**

Mina Ebrahimi-Rad<sup>\*1</sup>, Fatemeh Mirkhanim<sup>2</sup>, Mohammadmehdi Emam<sup>3</sup>, and Reza Saghiri<sup>1</sup>

<sup>1</sup>Biochemistry Department, Pasteur Institute of Iran, Tehran, Iran

<sup>2</sup>Department of Science, Parand Branch, Islamic Azad University, Parand, Iran

<sup>3</sup>Rheumatology Department, Loghman Hakim Hospital, Shaheed Beheshti University of Medical Sciences, Tehran, Iran

<sup>4</sup>Biochemistry Department, Pasteur Institute of Iran, Tehran, Iran

Osteoporosis is a growing public health problem in the most developed countries and a common age-related disease with low bone mass density (BMD) and increased fracture risk. Some of the fractures lead to morbidity and mortality especially in women. Many different factors are involved in osteoporosis pathogenesis, among which, genetic markers have been found to have a great impact (75-80%) on bone turn over and loss, predisposing to osteoporosis. Several genes have been studied as candidate genes in osteoporosis pathogenesis, including *COL1A1*, coding for the major bone matrix protein. This study was designed to analyze the correlation between the polymorphism of *COL1A1* gene regulatory upstream site at promoter region (-1997 G/T) and BMD variation in post-menopausal women. 424 Iranian post-menopausal women in the age range of 45-67 years old were enrolled into the study, considering the inclusion and exclusion criteria. Control healthy group (N:211, T-score  $\geq -1$ ) and patient group (N:213), including osteoporotic (T-score  $\leq -2.5$ ) and severely osteopenic (T-score  $> -2.5$ ) were analyzed. The decisions were made based on clinical examinations, BMD measurements by Dual-energy X-ray Absorptiometry (DXA), and the data obtained through a questionnaire, consisting of the necessary data. The subjects were genotyped by using PCR-Fragment Length Polymorphism (PCR-RFLP). Software Stata version 11 was applied to statistically analyze the collected data. The association between the specific genotypes, the low BMD and the disease was analyzed by using One-way ANOVA for quantitative variables. Although, for qualitative variables, test was applied in which  $P \leq 0.05$  was considered to be significant. Our data showed that there was no significant linkage between the -1997 G/T polymorphism in the *COL1A1* upstream regulatory region with the variation of bone mineral density (BMD) in Iranian postmenopausal women (for ee genotype versus EE+Ee the OR was 0.8, with 95% confidence CI was found to be 0.18-1.67).

## **P-027: Genetic control of extreme Influenza disease**

Martin T Ferris<sup>\*1</sup>, Kelsey E Noll<sup>1</sup>, Clayton R Morrison<sup>1</sup>, Ande West<sup>1</sup>, Alan C Whitmore<sup>1</sup>, Richard R Green<sup>2</sup>, and Mark T Heise<sup>1</sup>

<sup>1</sup>Department of Genetics, University of North Carolina at Chapel Hill

<sup>2</sup>Department of Microbiology, University of Washington, Seattle WA

Genetic variation within immune pathways has increasingly been seen as a critical player in a variety of human disease conditions. To understand how this variation in immune response pathways drives viral disease response to multiple respiratory diseases, we have used F1 crosses of the Collaborative Cross recombinant inbred panel. We have identified a wide range of immune response phenotypes to a number of pathogens, including Influenza A Virus. Importantly, we have identified a CC line, CC017/UNC that shows extreme susceptibility to the human pandemic A/California/04/09 virus. To better understand the underlying host pathways driving this extreme response, we have conducted in depth phenotyping of CC017/UNC and the classic inbred strain DBA/2J, which is also highly susceptible to influenza; furthermore we have generated and phenotyped an F2 cross between CC017/UNC and DBA/2J. We found that while C57BL/6J animals show a transient weight loss and >85% survival through 12 days post infection, CC017/UNC and DBA/2J strains show 100% mortality between 5-7 days post infection, with divergent disease etiologies. Furthermore, at 4 days post infection, all 3 strains show an influx of immune cells into the lungs, but there are strain-specific differences in neutrophil and t-cell populations following infection. Transcriptional analysis further confirmed an upregulation of phagocytic and granulocytic associated responses in both DBA/2J and CC017/UNC following infection. The F2 mapping population displays a wide range of Influenza response phenotypes, including animals that survive with only mild disease signs; a phenotype not seen within the parent strains.

**P-028: The environmental toxicant Tetrabromobisphenol-A promotes adipogenesis by downregulating THY1 (CD90) in human mesenchymal stem cells.**

E<sup>1</sup>Lissa Flores<sup>\*1,2,4</sup>, Collynn Woeller<sup>2,4</sup>, and Richard Phipps<sup>2,3,4</sup>

<sup>1</sup>Translational Biomedical Science, Clinical and Translational Institute

<sup>2</sup>Department of Environmental Medicine

<sup>3</sup>Department of Microbiology and Immunology

<sup>4</sup>University of Rochester, Rochester, NY

Obesity is characterized by excessive formation of adipocytes (adipogenesis) and increases in adipocyte size. Adipocytes produce pro-inflammatory adipokines and cytokines such as IL6 that promote the inflammatory state seen in obesity. Environmental factors are emerging as a cause of the drastic rise in obesity over the past decades and these require further study. Tetrabromobisphenol-A (TBBPA) is an environmental toxicant now called an "obesogen" as it can disrupt the endocrine system and increase adipogenesis. The mechanisms of action whereby TBBPA and other environmental toxicants promote fat cell formation and obesity remain unclear. THY1 (also called CD90) is a cell surface protein. THY1 has been widely used as a cell surface marker and is expressed on various subsets of cells, such as fibroblasts and stem cells. We have previously shown that only THY1<sup>-/low</sup> fibroblasts can differentiate into adipocytes while THY1<sup>+/high</sup> fibroblasts cannot, indicating THY1 can determine cell fate and is thus more than a cell marker. We hypothesized that TBBPA promotes adipogenesis by decreasing THY1 expression on human mesenchymal stem cells (hMSCs). Our data show that TBBPA promotes adipogenesis and decreases *THY1* mRNA and protein levels in hMSCs. Furthermore, TBBPA also reduces cell surface expression of THY1 in hMSCs. Adipogenesis was measured by visualization of lipid droplets and expression of the key adipocyte marker, fatty acid binding protein 4 (FABP4). Interestingly, even transient TBBPA exposure in hMSCs resulted in continued reduction of THY1 expression, even after TBBPA exposure was stopped. Therefore, developmental or even transient exposure to TBBPA could affect THY1 levels on stem cells and alter physiology long after exposure, which may contribute to the obesity epidemic.

## P-029: Genetics of Meiotic Recombination Rate

Barbora Faltusova<sup>1</sup>, Vaclav Gergelits<sup>1</sup>, Maria Balcova<sup>1</sup>, Tanmoy Bhattacharyya<sup>1,2</sup>, Ondrej Mihola<sup>1</sup>, Corrina Knopf<sup>1,3</sup>,  
Vladana Fotopulosova<sup>1</sup>, Zdenek Trachtulec<sup>1</sup>, Sona Gregorova<sup>1</sup>, and Jiri Forejt<sup>\*1</sup>

<sup>1</sup>Institute of Molecular Genetics AS CR, Division Biocev, Prague, Czech Republic

<sup>2</sup>Current address: The Jackson Laboratory, Bar Harbor, Maine, USA

<sup>3</sup>Current address: Medical University Vienna Center of Medical Physics and Biomedical Engineering, Vienna, Austria

During differentiation of germ cells into gametes, a maternal and a paternal copy of each chromosome have to find each other, recombine, pair and synapse in order to ensure proper chromosome segregation into the gametes. Because of the unique ability to identify homologous DNA sequences between homologous chromosomes, meiotic recombination is an essential step in proper chromosome pairing and synapsis in the majority of species. However, when the paternal and maternal sets of chromosomes come from different (sub)species, the recognition of homologs can be disturbed and result in sterility of male hybrids. Here we investigated the meiotic recombination rate at the global genome-wide level and its possible relation to hybrid sterility in mouse strains derived from subspecies *Mus m. musculus* (*Mmm*) and *Mus m. domesticus* (*Mmd*). Using immunofluorescence microscopy we quantified the foci of MLH1 DNA mismatch repair protein, the cytological counterparts of reciprocal crossovers, in a panel of inter-subspecific chromosome substitution strains. Two autosomes, Chr 7 and Chr 11, significantly modified the meiotic recombination rate, but the strongest modifier, designated meiotic recombination 1, *Meir1*, emerged in the 4.7 Mb *Hstx2* genomic locus on Chr X. Mapping *Meir1* to a narrow candidate interval on Chr X is an important first step towards positional cloning of the respective gene responsible for variation in the global recombination rate between closely related mouse subspecies.

## **P-030: Meiotic Arrest, Crossover Patterns, and Hybrid Sterility of [BALB/cJxJF1/Ms] F<sub>1</sub> Male Mice**

Yasuhiro Fujiwara<sup>\*1,2</sup>, Risako Nishino<sup>2,3</sup>, Tanmoy Bhattacharyya<sup>1</sup>, Petko M Petkov<sup>1</sup>, Tetsuo Kunieda<sup>4</sup>, and Mary Ann Handel<sup>1</sup>

<sup>1</sup>The Jackson Laboratory, Bar Harbor, ME 04609

<sup>2</sup>Graduate School of Natural Science and Technology, Okayama University, Okayama University, Okayama, Okayama 700-8530, Japan

<sup>3</sup>The Institute of Environmental Toxicology, Joso, Ibaraki, 303-0043, Japan

<sup>4</sup>Graduate School of Environmental and Life Science, Okayama University, Okayama, Okayama 700-8530, Japan

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Organisms that belong to different subspecies within the same species sometimes exhibit reproductive isolation owing to failure in gametogenesis, and hybrid sterility (HS) of F<sub>1</sub> offspring. Here, we investigate the basis of HS mechanism found in intersubspecific F<sub>1</sub> hybrid mice produced by matings between *Mus m. domesticus* (*domesticus*) classical laboratory mouse strains and *Mus m. molossinus* (*molossinus*) Asian mouse strains. We found that F<sub>1</sub> (designated CJFF1) males from a mating between female BALB/cJ mouse (*domesticus*) and male Japanese fancy mouse JF1/Ms (*molossinus*) were sterile, although the F<sub>1</sub> females were fertile, and the reciprocal F<sub>1</sub> males (designated JFCF1) were sub-fertile. Sterility of the CJFF1 males was due to high rate of arrest during the first meiotic division phase. Pachytene spermatocytes of the CJFF1 males exhibited a high rate of dissociation of the XY Chromosomes, and metaphase spermatocytes showed dissociation of the XY Chromosomes as well as premature separation of autosomal chromosomes. Although many aspects of recombination-related DSB formation and repair were similar between sterile and sub-fertile F<sub>1</sub> males, patterns of crossing over were atypical in the CJFF1 males. We therefore infer that incompatibilities between the BALB/cJ and JF1/Ms subspecies, manifest asymmetrically in their F<sub>1</sub> males, affect multiple aspects of meiosis, culminating in arrest of the division phase. Supported in part by NIH HD33816 to MAH.

**P-031: ENU mutation cataloging in the RINEN ENU mutant mouse library using whole exome analysis with Ion Proton sequencer.**

Ryutaro Fukumura<sup>\*</sup>, Hayato Kotaki, Shigeru Makino, Yuichi Ishitsuka, Yuji Nakai, and Yoichi Gondo

Mutagenesis and Genomics Team,RIKEN BRC

We have been providing allelic series of mutant mice carrying a single point mutation in the target gene as a public resource named "RIKEN ENU-based gene-driven mutagenesis system (RGDMS). Since we find the base substitution first, it is very easy to phenotype not only the heterozygotes but also homozygotes. The KO mice become available from the IKMC or by the genome editing. The CRISPR/Cas9 system is also able to provide targeted base substitutions if we know which base pair to be substituted before hands. Thus, when target base-pair(s) are not known, the RGDMS is more effective because it provide about 10 allelic series of base-substitutions in the target gene. In addition, each G1 mouse carries ~5,000 base substitutions that provide a resource to detect and analyze the genetic interactions and polygenic functions among them.

We have started the whole exome sequencing (WES) for G1 genome in order to comprehensively detect SNV induced by ENU. We have so far discovered more than 3000 SNVs by using SOLiD4 and HiSEQ2000. Here, we report the WES study using Ion Proton sequencer. We obtained ~80 million reads from each G1 mouse per PI chip. The realignment BAM files mapped on GRCh38/mm10 were made by Torrent server. Using the MassARRAY or the amplicon sequencing, we validated the SNV candidates that may have a significant effect on the protein sequences, such as missense or nonsense mutations. As a result, we newly extracted 1207 SNV candidates on the 1124 genes from 27 G1 genomes. From OMIM database, 237 genes were related to the 525 diseases. We experimentally validated the 246 SNV candidates from 5 G1 genomes and found that 246 (100%) were true mutations. By making the catalog of the ENU mutations, our system should contribute to understand the mechanism of the genome functions with polygenic disorders.



## P-032: Correlation of *Trp53cor1* and *Trp53* expression in the *Trp53cor1* gene trap mouse line

Riki Furuhashi<sup>1</sup>, Mai Nakahara<sup>1</sup>, Haruka Ito<sup>1</sup>, Masatake Araki<sup>2</sup>, Kumiko Yoshinobu<sup>2</sup>, Ken-ichi Yamamura<sup>3</sup>, and Kimi Araki<sup>1</sup>

<sup>1</sup>Division of Developmental Genetics, IRDA, Kumamoto University

<sup>2</sup>Division of Bioinformatics, IRDA, Kumamoto University

<sup>3</sup>Yamamura Project Laboratory, IRDA, Kumamoto University

*Trp53cor1* (*LincRNA-p21*) was recently identified as a long intergenic non-coding RNA (lincRNA) regulated by TRP53, and reported to function in TRP53-dependent repression of target genes and induction of apoptosis (Huarte et al, Cell 142, 409-419). Many other interesting functions of *Trp53cor1* have been reported, such as regulation of Warburg effect by promoting glycolysis under hypoxia, transcriptional activation of the neighboring *Cdkn1a* (*p21*) gene in *cis*. Most studies of *Trp53cor1* were performed using culture cells, therefore, the *in vivo* function of *Trp53cor1* is unclear. We found one of our exchangeable gene trap clones, Ayu21-B186, has trapped the *Trp53cor1* gene and established the *Trp53cor1* gene trap mouse line B6.Cg-*Trp53cor1*<sup>Gt(Ayu21-B186)Imeg</sup> to analyze its functions in mice.

The trap vector was inserted in the intron at 12kb downstream of the 1st exon, and the fusion transcript of the 1st exon and the *lacZ* gene was produced. To examine the expression pattern of *Trp53cor1* in mice, RT-PCR and X-gal staining of adult tissues were performed. Although RT-PCR analysis revealed that *Trp53cor1* was weakly expressed in almost all mouse tissues examined, relatively strong X-gal staining was detected in the cerebellum and pancreas. In order to know whether *Trp53cor1* is induced by TRP53 *in vivo*, we crossed B6.Cg-*Trp53cor1*<sup>Gt(Ayu21-B186)Imeg</sup> gene trap line to TRP53-deficient (B6.Cg-*Trp53*<sup>tm1Sia</sup>) mice. In B6.Cg-*Trp53*<sup>tm1Sia</sup>/*Trp53*<sup>tm1Sia</sup> *Trp53cor1*<sup>Gt(Ayu21-B186)Imeg</sup>/+ mice, X-gal staining in the cerebellum and pancreas completely disappeared. Next, we induced TRP53 expression through high-fat diet and experimental pancreatitis and examined the *Trp53cor1* expression. In the TRP53 up-regulated tissues, the *Trp53cor1* was also highly expressed. These results indicate that *Trp53cor1* is regulated by TRP53 *in vivo*.

**P-033: Maternal malnutrition alters gene expression, genomic methylation and behavioral phenotypes of progeny**

Tamio Furuse<sup>\*1</sup>, Takashi Kohda<sup>2</sup>, Kunio Miyake<sup>3</sup>, Takae Hirasawa<sup>4</sup>, Tomoko Kushida<sup>1</sup>, Ikuko Yamada<sup>1</sup>, Misho Kashimura<sup>1</sup>, Hideki Kaneda<sup>1</sup>, Kimio Kobayashi<sup>1</sup>, Fumitoshi Ishino<sup>2</sup>, Takeo Kubota<sup>3</sup>, and Shigeharu Wakana<sup>1</sup>

<sup>1</sup>Japan mouse clinic, RIKEN BRC, Japan

<sup>2</sup>Dept. of Epigenetics, Tokyo Med. & Dent. Univ., Japan

<sup>3</sup>Dept. of Epigenetic Med., Univ. of Yamanashi, Japan

<sup>4</sup>Dept. of Bioscience., Teikyo University, Japan

The developmental origins of health and disease paradigm (DOHaD) is a concept that fetal environmental factors affect the adult phenotypes. We have been performing study that validates the DOHaD theory in the pathogenesis of psychiatric disorders and developmental disorders using mouse models. In this study, *in vitro* fertilization (IVF) and embryo transfer (ET) technics were used for mouse reproduction. ICR female mice were used as recipient and foster mothers. The embryos were prepared from eggs and sperms of C57BL/6J. We provided following diets as experimental diets to pre-pregnant and pregnant females, AIN93G (control diet, CD), low-protein (LP), and low-protein diet that contains folate supplement (LP + FA). The embryos obtained from C57BL/6J were transferred to the recipient mothers. The offspring which were exposed to malnutrition *in utero* exhibited increased activity in the home cage, decreased contact to novel object, and decreased social investigation. The adult offspring of LP group and LP+FA group exhibited different pattern of mRNA expression and genomic methylation in the brain. In addition, we are developing new DOHaD model by using maternal mice which carry mutations in genes that relate to nutrient transport. In this meeting, we will report about the summary of past study and progress of the development of new model.

**P-034: Transcriptional control of hibernation: first insights on comparative genomics of dormice**

(See abstract TS-03 in the Trainee Symposium)

**P-035: Conservative miRNA target analysis: are we limiting our discoveries of neuronal miRNA function?**

(See abstract TS-06 in the Trainee Symposium)

### **P-036: Needs of fundamental revision of mouse genome reference sequences.**

Yoichi Gondo<sup>\*1</sup>, Kazuki Mori<sup>2</sup>, Ryutarō Fukumura<sup>1</sup>, Atsushi Toyoda<sup>3</sup>, Satoshi Oota<sup>1</sup>, Toyoyuki Takada<sup>4</sup>, Ryutarō Himeno<sup>5</sup>,  
Toshihiko Shiroishi<sup>4</sup>, Satoru Kuhara<sup>2</sup>, and Asao Fujiyama<sup>3</sup>

<sup>1</sup>RIKEN BioResource Ctr

<sup>2</sup>Grad. Sch. Agr., Kyushu Univ.

<sup>3</sup>Comp. Genome, Natnl. Inst. Genet.

<sup>4</sup>Mamm. Genet., Natnl. Inst. Genet.

<sup>5</sup>RIKEN Adv. Ctr. Comp. Comm.

Chaisson et al. [1] and Pendleton et al. [2] have conducted re-assembly of whole human genome reference sequences by using long-read WGS this year. The gaps and whole structural variations (SVs) have been significantly closed and revised, respectively. The mouse genome reference sequences have been considered to be more precise than human's, since they are from C57BL/6J inbred genomic DNA. Fairfield et al. [3] reported that 50% of Mendelian traits in the mutant mouse strains were identified by WES, the efficiency of which was very equivalent to those in human. It implies that the mouse genome reference sequences also have some unexpected issues in addition to those found in human genome reference sequences. We have conducted WGS of C57BL/6Jcl with >500-fold depth and found peculiar SNVs as well as SNPs, suggesting that not only the unfinished gaps but also the SV issues must be solved in the mouse genome reference sequences as well. The re-assessment and re-assembly of mouse genome reference sequences seems to be urgent and necessary, since the designing of PCR primers and genome editings are all relied on the genome reference sequences. Not only the re-sequencing to find de novo mutations and SNPs but also RNAseq, ChIPseq, Egig genome-seq, etc. are also depending on the genome reference sequences.

1. Chaisson et al. Nature 517: 608-611, 2015.

2. Pendleton et al. Nat Method 12: 780-786, 2015.

3. Fairfield et al. Genome Res 25: 948-957, 2015.

**P-037: Mapping SARS-Coronavirus susceptibility alleles using the Collaborative Cross**

(See abstract TS-02 in the Trainee Symposium)

**P-038: SINEUPs: a new class of natural and synthetic antisense long non-coding RNAs that activate translation.**

Silvia Zucchelli<sup>1,2</sup>, Diego Cotella<sup>2</sup>, Hazuki Takahashi<sup>3</sup>, Francesca Fasolo<sup>1</sup>, Michael Jones<sup>4</sup>, Francesca Persichetti<sup>2</sup>, Remo Sanges<sup>5</sup>, Claudio Santoro<sup>2</sup>, Piero Carninci<sup>3,6</sup>, and Stefano Gustincich<sup>\*1,6,7</sup>

<sup>1</sup>Area of Neuroscience, SISSA, Trieste, Italy

<sup>2</sup>Dipartimento di Scienze della Salute, Università del Piemonte Orientale, Novara, Italy

<sup>3</sup>RIKEN Center for Life Science Technologies, Division of Genomic Technologies, Yokohama, Japan

<sup>4</sup>Cell Guidance Systems, Cambridge, United Kingdom

<sup>5</sup>Animal Physiology and Evolution Laboratory, Stazione Zoologica Anton Dorn, Napoli, Italy

<sup>6</sup>TransSINE Technologies, Yokohama, Japan

<sup>7</sup>Department of Neuroscience and Brain Technologies, Italian Institute of Technology (IIT), Genova, Italy.

We present the discovery of a new functional class of natural and synthetic antisense lncRNAs that stimulate translation of sense mRNAs. These molecules have been named SINEUPs since their function requires the activity of an embedded inverted SINEB2 sequence to UP-regulate translation. Natural SINEUPs suggest that embedded Transposable Elements may represent functional domains in long non-coding RNAs. Synthetic SINEUPs may be designed by targeting the antisense sequence to the mRNA of choice representing the first scalable tool to increase protein synthesis of potentially any gene of interest.

We will discuss potential applications of SINEUP technology in the field of molecular biology experiments, in protein manufacturing as well as in therapy of haploinsufficiencies.

**P-039: Genome-wide mapping of hyper-acetylated chromatin with a novel antibody in lung cancer cells**

Lusy Handoko<sup>\*1</sup>, Marina Lizio<sup>1</sup>, Wakamori Masatoshi<sup>2</sup>, Michiel De Hoon<sup>1</sup>, Aki Minoda<sup>1</sup>, and Takashi Umehara<sup>2,3</sup>

<sup>1</sup>Division of Genomic Technologies, RIKEN CLST

<sup>2</sup>Epigenetic Drug Discovery Unit, Division of Structural and Synthetic Biology, RIKEN CLST

<sup>3</sup>PRESTO, JST

Changes in acetylation of histone H4 are a common hallmark of cancer cells. In leukemia cells, histone H4 is characterized by a loss of K16 mono-acetylation. Bromodomain proteins, implicated in cancer, specifically recognize acetylated lysines. Recently, inhibitors of DNER(BET)-bromodomain (JQ-1 and I-BET) have been developed as promising anti-cancer agents. These indicate prominent roles of H4-acetylation in transcriptional regulation in cancer cells. Less study, however, has been focused on histone H4-acetylation at a **genome-wide level**. Here, we aim to profile histone H4-acetylation sites by CHIP-seq in lung cancer cells. With a novel monoclonal antibody we generated, we found around 70% of hyper-acetylated H4 is associated with active enhancers. Furthermore, a small subset of hyper-acetylated H4 is associated with super-enhancers. Incorporating the genome-wide H4-acetylation patterns with other epigenetic state marks (enhancers, repressors and transcription factors BRDs) will give us insight into the role of histone H4 acetylation in the epigenetic regulation of lung cancer cells



## **P-040: Up-regulation of non-coding RNAs in adult and pediatric liver cancers**

Kosuke Hashimoto<sup>\*1</sup>, Ana Maria Suzuki<sup>1</sup>, Alexandre Dos Santos<sup>2</sup>, Christophe Desterke<sup>2</sup>, Emilie Braun<sup>2</sup>, Alessandro Bonetti<sup>1</sup>, Alexandre Fort<sup>1</sup>, Xian-Yang Qin<sup>1</sup>, Bogumil Kaczkowski<sup>1</sup>, Alistair Forrest<sup>1</sup>, Soichi Kojima<sup>1</sup>, Didier Samuel<sup>2</sup>, Marie Annick Buendia<sup>2</sup>, Jamila Faivre<sup>2</sup>, and Piero Carninci<sup>1</sup>

<sup>1</sup>RIKEN Center for Life Science Technologies

<sup>2</sup>INSERM U785, Centre Hepatobiliaire

An increasing number of non-coding RNAs (ncRNAs) are implicated in various human diseases including cancer, however ncRNA transcriptome of liver cancer remains largely unexplored. We use CAGE to comprehensively map transcription start sites (TSSs) and measure their expression in two types of liver cancers, human hepatocellular carcinoma and hepatoblastoma with special emphasis on ncRNAs. We found thousands of significantly up-regulated ncRNAs in both types of liver cancers compared to their matched non-tumors. In HCC, many LTR retroviral promoters are activated in a subfamily-specific manner. Intriguingly, the expression of highly up-regulated LTRs tends to be limited in reproductive tissues, such as testis and placenta. 3' RACE for 15 highly up-regulated LTRs revealed that the transcripts are multi-exon ncRNAs typically 0.5-2kb in length. On the other hand, hepatoblastoma didn't show strong activation of LTR promoters. Instead, we identified activated ncRNAs that have TCF/LEF binding motifs, which are likely to be targets of the wnt/ $\beta$ -catenin pathway. Together, this study sheds light on ncRNA transcriptome of liver cancers, which might play important roles in tumor progression.

**P-041: Dissecting the contribution of genes from the 17q21.31 region in the Koolen-deVries deletion syndrome using the mouse**

Thomas Arbogast<sup>1</sup>, Hamid Meziane<sup>2</sup>, Giovanni Iacono<sup>3</sup>, Arnaud Duchon<sup>1</sup>, Mohammed Selloum<sup>2</sup>, Marie-Christine Birling<sup>2</sup>, Tania Sorg<sup>2</sup>, Henk G. Stunnenberg<sup>3</sup>, the Gencodys Network, Hans van Bokhoven<sup>3</sup>, David Koolen<sup>3</sup>, Bert de Vries<sup>3</sup>, and Yann Herault<sup>1,2</sup>

<sup>1</sup>Institut Clinique de la Souris, PHENOMIN-ICS, CNRS, INSERM, Université de Strasbourg, 1 rue Laurent Fries, 67404 Illkirch, France

<sup>2</sup>Institut de Genétique Biologie Moléculaire et Cellulaire, IGBMC, CNRS, INSERM, Université de Strasbourg, UMR7104, UMR964, 1 rue Laurent Fries, 67404 Illkirch, France

<sup>3</sup>Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands

Genetic diseases with intellectual disability (ID) can occur during the developmental period and is defined by an intellectual quotient below 70. Several genetic causes, including Down syndrome, deletion or duplication of genomic regions, and more than 500 genes, have been associated with ID. To better understand the pathophysiology of the 17q21.31 Koolen deVries syndromes (KdVS), characterized by a deletion of a short genomic region, we generated a series of mouse mutants including the deletion and the duplication of the homologous region, and the inactivation of candidate genes. Here we will report the characterization of mouse models using standardized behavioural and cognitive paradigms. Based on the new series of models and further studies, we identified one candidate gene as the main contributor to the syndrome having a smaller contribution to the disease. In addition we went further and we were able to identify gene networks and chromatin changes in the models. The data generated are challenging our current knowledge and offer new perspectives for a better understanding of the KdV syndrome and its future treatment.

**P-042: Effects of early-life exposure to Western diet on adult activity levels and associated behavioral and physiological traits in mice**

Layla Hiramatsu<sup>\*</sup>, Ralph Albuquerque, Gerald Claghorn, Jarren Kay, Jennifer Singleton, Zoe Thompson, James Colbath, Brett Ho, Brittany Ho, Gabriela Sanchez-Andrade, Danny Thai, and Theodore Garland Jr.

University of California Riverside, Riverside, CA 92521

Lack of physical activity contributes to many human diseases, as well as overweight and obesity. Numerous factors that influence activity levels, genetic or environmental, may occur during development and early life of an individual. Using an ongoing selection experiment with 4 replicate lines of mice bred for high voluntary wheel running (and 4 replicate, non-selected control lines), 100 female mice were given a "Western diet" (WD) with increased fat and added sucrose, while an additional 100 female mice were given standard diet (SD), from 2 weeks prior to mating until their pups could feed on solid food (~14 days of age). Of the resulting pups, 100 males (50 WD and 50 SD) were considered focal mice and received various tests: behavioral spot checks from birth, elevated plus maze, VO<sub>2</sub>max during forced treadmill exercise, 6 days of wheel access (with additional mice), home-cage activity, body fat composition, and organ masses. Nested ANCOVA was used with line nested within linetype (high-runner or control), with body mass and/or age as covariates. Exposure to WD during early life had a significant positive effect on the adult body mass of males for both HR and C lines, and caused a diet-by-line interaction in HR females. Early-life WD increased wheel running in C males, and caused a diet-by-line interaction in HR females. As expected from previous studies, HR mice had higher VO<sub>2</sub>max than C, but VO<sub>2</sub>max was unaffected by WD. Heart ventricle mass was increased by early-life WD in both HR and C mice. Interestingly, early-life WD did not increase fat pad masses. Analyses of additional behavioral and physiological traits are ongoing. Supported by US NSF grant IOS-1121273 to TG.

**P-043: An atlas of 5' complete transcripts reveals the genomic origins and expression landscape of human long non-coding RNAs**

Chung-Chau Hon<sup>\*1</sup>, Jordan A Ramilowski<sup>1</sup>, The FANTOM Consortium<sup>1</sup>, Yoshihide Hayashizaki<sup>2</sup>, Piero Carninci<sup>1</sup>, and Alistair Forrest<sup>1,3</sup>

<sup>1</sup>RIKEN Center for Life Science Technologies (Division of Genomic Technologies), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 Japan

<sup>2</sup>RIKEN Preventive Medicine and Diagnosis Innovation Program, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

<sup>3</sup>Harry Perkins Institute of Medical Research, QEII Medical Centre and Centre for Medical Research, the University of Western Australia, Nedlands, Western Australia, Australia

There is a strong controversy over the fraction of long non-coding RNA (lncRNAs) that are products of regulated and functional, versus spurious and non-functional, transcription events. One aspect hampering their study is the incomplete transcript models assembled from short reads, leading to misidentification of their 5' ends and hence their regulatory regions. Herein we applied Cap Analysis of Gene Expression (CAGE) to identify the 5' ends of lncRNA transcript models from various sources. Overlaying these 5' complete models onto DNase I hypersensitivity sites (DHS), we defined the putative regulatory regions (e.g. promoter or enhancer) controlling their transcription. Epigenetic marks indicate that the majority of intergenic lncRNAs are derived from enhancer-like regions (e-lncRNAs). These regulatory regions, and sometimes the RNA stretches outside of the DHS, showed evidence of non-neutral selection, and were significantly enriched in GWAS and eQTL associated SNPs. Using the expression profiles of lncRNAs measured by the FANTOM5 atlas, we show that those that overlap an eQTL associated SNP are significantly more correlated with the expression of their eQTL associated mRNA partner than random pairs of lncRNAs and mRNAs at similar distances, providing multiple layers of evidence supporting coordinated regulation of lncRNA and mRNA loci. Lastly, using sample ontology enrichment and differential expression analyses, we annotate these transcripts with the cell types in which they are enriched in and the physiological conditions in which they are dynamically regulated, summarizing these annotations in a web resource. This forms the foundation for a new effort to functionally characterize lncRNAs in the genome.

**P-044: CAGE revealed novel biomarker of periodontitis-associated fibroblasts**

Masafumi Horie<sup>\*1,2,3</sup>, Yoko Yamaguchi<sup>4</sup>, Akira Saito<sup>1,2</sup>, Takahide Nagase<sup>1</sup>, Masayoshi Itoh<sup>3,5</sup>, Hideya Kawaji<sup>3,5</sup>, Timo Lassmann<sup>3,5</sup>,  
Piero Carninci<sup>3,5</sup>, Yoshihide Hayashizaki<sup>6</sup>, Alistair Forrest<sup>3,5</sup>, The FANTOM consortium, Marina Lizio<sup>3,5</sup>, Masahiro Kondo<sup>7</sup>,  
Tatsuo Suzutani<sup>8</sup>, Patrick Micke<sup>9</sup>, and Mitsuhiro Ohshima<sup>10</sup>

<sup>1</sup>Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo

<sup>2</sup>Division for Health Service Promotion, The University of Tokyo

<sup>3</sup>Division of Genomic Technologies (DGT), RIKEN Center for Life Science Technologies

<sup>4</sup>Department of Biochemistry, Nihon University School of Dentistry

<sup>5</sup>Omics Science Center, RIKEN Yokohama Institute,

<sup>6</sup>RIKEN Preventive Medici

