Plenary Speakers

Abstracts PL1 – PL8

PLENARY PRESENTATION – PL1 MONDAY NOVEMBER, 3 8.30AM – 9.00AM

EPIGENETIC REGULATION OF LINEAGE COMMITMENT AND PLURIPOTENCY IN THE MAMMALIAN EMBRYO

R Ng, C Farthing, G Ficz, F Santos, S Andrews, C Popp, W Dean, M Hemberger & W Reik
Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge CB22 3AT, University of Cambridge, UK

Epigenetic reprogramming and lineage specific acquisition of epigenetic marking occurs in the mammalian germline and in preimplantation embryos. Active and passive demethylation is followed by de novo methylation which preferentially occurs in inner cell mass cells in the blastocyst. We have begun to carry out genome wide profiling studies of various pluripotent, multipotent, and terminally committed celltypes in the germline and the early embryo. We have identified various classes of genes, such as regulators of pluripotency, which are differentially methylated and expressed in pluripotent cell types as compared to more differentiated ones. This list of genes includes new candidates for the regulation of pluripotency and reprogramming which need to be functionally tested.

We have begun to test the hypothesis that epigenetic regulation is important for lineage commitment and its stability. Wildtype ES cells do not normally differentiate into cells of the trophectoderm lineage, while we find that ES cells deficient in DNA methylation do so efficiently. Using our screen we identified a trophoblast transcription factor that is methylated and repressed in ES cells, but unmethylated and expressed in TS cells. Expression of this transcription factor sustains a positive feedback loop between key trophoblast fate determinants, and is thus necessary for trophoblast commitment. By contrast, epigenetic silencing of this trophoblast determinant in the epiblast lineage disrupts the feedback loop so that trophoblast cell fate is aborted should it initially take place through stochastic expression of some transcription factors. This type of epigenetically regulated feedback loop appears to ensure initial plasticity followed by canalization of lineage fate.

Finally, we are studying the mechanisms of demethylation in the germline and the early embryo, which are critical for the transmission of pluripotency.

PLENARY PRESENTATION – PL2 MONDAY NOVEMBER, 3 1.00PM – 1.30PM

HOW LITTLE WE KNOW: FUNCTION AND CONNECTIVITY OF THE GENOME

Stylianos E. Antonarakis
University of Geneva Medical School, Geneva, Switzerland

The deciphering of the function of the mammalian genome is a formidable challenge, but a necessary step in order to understand the molecular mechanisms of the numerous phenotypic traits including the human disorders. Not only all functional elements need to be identified, but also the functional variability of the genome needs to be recognized. Data on conserved non-coding sequences, and their connectivity to different *cis* and *trans* genomic regions will be presented. In addition, the genic landscape of the genome will be discussed.

VERNE CHAPMAN MEMORIAL LECTURE – PL3 MONDAY NOVEMBER, 3 4.30PM – 5.30PM

FACETS OF X-INACTIVATION: EXPLORING AN EPIGENETIC PARADIGM

Philip Avner
Developmental Biology Dept, Mouse Molecular Genetics Unit, Institut Pasteur, Paris, France

X-inactivation is a highly regulated process ensuring dosage compensation of X-linked genes in female mammals. In eutherian mammals, the initiation of X- inactivation is controlled by the X- inactivation center and involves the decoration of the presumptive inactive X chromosome by the non-coding Xist RNA, the recruitment of a series of repressive proteins and modifications to histone constituents making up the X chromosome. Recent progress in our knowledge of the X-inactivation process will be presented and insights into its complexities explored.

PLENARY PRESENTATION – PL4 TUESDAY NOVEMBER, 4 8.30AM – 9.00AM

PROGRESS AND PROSPECTS IN RAT GENETICS – IDENTIFICATION OF DISEASE GENES, PATHWAYS AND MECHANISMS

Timothy J Aitman
MRC Clinical Sciences Centre and Imperial College, London, United Kingdom

The rat is an important system for modeling human disease. Four years ago, the history of rat research was transformed by the sequencing of the rat genome, ushering in an era of exceptional opportunity for identifying genes and pathways underlying disease phenotypes. Genome-wide association studies in human populations have recently provided a direct approach for finding robust genetic associations in common diseases, but identifying the precise genes and their mechanisms of action remains problematic. In the context of significant progress in rat genomic resources over the past decade, this talk will outline achievements in rat gene discovery to date, will show how these findings have been translated to human disease, and will describe how an expanding community of rat geneticists are driving an increasing pace of discovery of new disease genes, pathways and mechanisms in the rat model.

PLENARY PRESENTATION – PL5 TUESDAY NOVEMBER, 4 1.00PM – 1.30PM

STUDYING THE GENETIC BASIS OF ADAPTATIONS IN WILD MOUSE POPULATIONS

Diethard Tautz
Max-Planck Institute for evolutionary Biology, Plön, Germany

A fundamental question of evolutionary bioloy is the frequency of adaptive changes and the nature of the genes and mutations involved in adaptations. Evolution is very unlikely to procede in major sudden steps. Instead, most mutations will cause only relatively small differences in fitness under natural conditions. This implies that they are difficult to detect by genetic experiments in the laboratory. One way of identifying the genetic basis of adaptations is to screen genomes for signatures of selection. Positive selection in a genomic region leaves a characteristic footprint behind, namely reduced neutral variation around the selected site (selective sweep). This can be employed to systematically screen for positively selected (adaptive) mutations and allows to estimate their frequency in a given population. Another way of finding adaptive trait loci is to assess differences between closely related species, e.g. via transcriptome analysis on microarrays. In my presentation I will present results from such screens in different populations of the house mouse and discuss the implications.

PLENARY PRESENTATION – PL6 WEDNESDAY NOVEMBER, 5 8.30AM – 9.00AM

PL6 - GENETIC ARCHITECTURE OF COMPLEX TRAITS IN THE MOUSE

Jonathan Flint
Wellcome Trust Centre for Human Genetics, Oxford, UK

Behaviour genetics in the mouse is at a turning point. In the next few years resources will become available to test the behavioural function of any gene in the genome, placing it within genetic, transcript and protein networks. Interpreting the new data will require a modification of the causal model in which genes are arranged in linear pathways. Instead, a systems biology approach will be necessary.

PLENARY PRESENTATION – PL7 WEDNESDAY NOVEMBER, 5 1.15PM – 1.45PM

INFERRING GENOME-WIDE ARCHITECTURE AND EVOLUTION OF GENE NETWORKS FROM CHIP-SEQ AND RNA-SEQ DATA

Brian Williams*, Ali Mortazavi*, Gordon Kwan, Anthony Kirilusha, Sandra Sharp, Katherine Fisher and Barbara Wold
California Institute of Technology, USA

To understand how gene networks function and how they have evolved, we first need to assemble comprehensive and accurate physical maps of network inputs and outputs. We are working to construct a complete genome-wide map of the network that directs skeletal muscle development in mouse, man and several other animals. These physical maps include thousands of binding sites for multiple transcription factors, both tissue specific and "general" ones; RNA Polymerase II occupancy measurements; plus the resulting mRNA output from the network. To make these measurements we have been developing a set of sequence-census assays and associated bioinformatics. They all take advantage of recently developed high throughput DNA sequencing (HTS) platforms: to map the relevant transcriptomes we sequenced deeply a cDNA copy of mRNA from myogenic mouse myogenic cells and from muscle tissue (20M- 80M reads/per transcriptome). The resulting transcriptome maps quantify mRNA abundance over a range of ~5 orders of magnitude, affording robust detection of transcripts (~1X sequence coverage) for RNAs present at concentrations of 1-5 copies per cell. RNA splice events, including alternate splicing, were identified by mapping sequence reads that cross known or theoretical splice junctions. We then used the previously developed ChIP-Seq (Johnson et al., 2007) to determine genomewide in vivo occupancy for transcription factors, RNA Pol2 isoforms and histone modification states. We discuss our initial conclusions from integrating these diverse datasets and raise some questions concerning evolution and function of the network.

PLENARY PRESENTATION – PL8 WEDNESDAY NOVEMBER, 5 3.00PM – 3.30PM

GENETICS AND IMAGING OF MURINE NEURAL TUBE CLOSURE

Lee Niswander and Christina Pyrgaki
University of Colorado Denver and HHMI, USA

The neural tube is the embryonic precursor of the central nervous system (CNS), the brain and spinal cord. The CNS tissue starts as a flat "plate" which then rolls up to form the neural tube. During neural closure, the neural cells are dividing, undergoing complex patterning to form the appropriate neuronal precursors, changing their shape, and interacting with new neighbors. Failure to close the neural tube results in neural tube defects, like spina bifida and exencephaly, the second most common human birth defect. However, little is known of the genes that control neural tube closure or how these genes work.

To gain insight into this complex morphogenetic process, we have used an unbiased approach of forward genetic screening in mice to identify a number of genes that are critically required for neural tube closure. Our goal is to clone the genes which when mutated cause neural tube defects and to determine the mechanisms by which they act to regulate this critical embryonic process. To date we have cloned 12 new genes necessary for neural tube closure. This is leading to novel insights into Hedgehog signaling, regulated proliferation, interactions between head mesenchyme and neural tissue, and neural fold fusion. As another approach, we are employing dynamic imaging of neural tube closure to study the normal events and to determine how cell behavior is disrupted in the various neural tube mutants. Our third and long-term goal is to understand how dietary supplements like folic acid help to prevent neural tube defects and to identify new therapies to correct folate-resistant neural tube defects.

ORAL PRESENTERS ABSTRACTS

O-2 - O-43

IMMUNITY/INFECTION/EPIGENETICS
ORAL PRESENTATION MONDAY NOVEMBER, 3 9.00AM – 9.15AM

O-2 CTCF AND COHESIN BIND AT THE BASE OF A CHROMATIN LOOP TO ISOLATE GENES THAT ESCAPE X INACTIVATION AND HAVE A DIFFERENT EPIGENETIC SIGNATURE ON THE MOUSE X CHROMOSOME

Fan Yang[1], Joel Berletch[1], Galina Filippova[2], Christine Disteche[1]
[1]Departments of Pathology and Medicine, University of Washington, Seattle, WA 98195, United States, [2]Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, United States

We have previously shown that genes that escape from X inactivation are separated from genes subject to silencing by transition regions that bind the chromatin boundary element CTCF. We have now shown that the cohesin complex, as detected by chromatin immunoprecipitation using an antibody for one member of the complex RAD21, also binds to such regions. Binding of CTCF and RAD21 was found both in male and female mouse adult liver at the 5'ends of the escape genes *Jarid1c* and *Eif2s3x*. Enrichment was higher in female mice, suggesting that CTCF together with RAD21 may play a role in escape from X inactivation. Our ChIP-chip survey of the distribution of methylation of lysine 27 at histone H3, a chromatin mark associated with X inactivation, showed that escape genes were completely devoid of this epigenetic mark. Furthermore, chromatin conformation capture (3C) assays demonstrated that a chromatin loop formed around the escape gene *Jarid1c*.

We propose that CTCF together with RAD21 play a specific role in escape from silencing, possibly through the formation of a chromatin loop fastened by the cohesin complex, which protects the chromatin region from histone modifications associated with silencing.

IMMUNITY/INFECTION/EPIGENETICS
ORAL PRESENTATION MONDAY NOVEMBER, 3 9.15AM – 9.30AM

O-3 FORWARD GENETIC ANALYSIS OF THE INNATE IMMUNE RESPONSE IN WILD-DERIVED MICE

James Conner, Irina Smirnova, Alexander Poltorak
Tufts University School of Medicine, Boston, MA, United States

The mammalian immune response is controlled by the integrated effects of numerous cytokines and their respective signaling pathways. The contribution of numerous effector molecules, produced in a time and concentration dependent manner, suggests that this system is highly adaptable and well evolved. Here we describe phenotypic characterization and QTL mapping of a non-canonical pattern of cytokine transcription in wild-derived mice, where genetic diversity has arisen in an evolutionary context. Activation of toll-like receptors (TLRs) in macrophages isolated from the wild derived strains MOLF/Ei, CZECH/EiII, and MSM/Ms resulted in rapid and significantly more robust transcription of IL-6, a pro-inflammatory cytokine traditionally ascribed to a "second wave," more delayed response in macrophages from classical inbred mice. In contrast, the canonically "first wave" cytokine TNFα displayed similar induction kinetics and accumulated to roughly equal levels in all strains tested, suggesting that wild-derived mice have specifically co-opted a more rapid IL-6 response under distinct evolutionary conditions. QTL mapping revealed one major and several minor determinants of the trait, many of which displayed highly significant epistatic interactions. shRNA knockdown of one candidate gene, *Irak2*, on the major locus resulted in abrogated IL-6 production in MOLF/Ei, but not C57BL/6J macrophages. Finally, a negatively acting locus detected in wild-derived strains contained a *cis*-acting regulatory element, allowing inducible expression of the inflammatory repressor IRAK1BP1, implying that mechanisms to protect against deleterious effects of excessive cytokine production have co-segregated with the trait of rapid IL-6 induction.

IMMUNITY/INFECTION/EPIGENETICS
ORAL PRESENTATION MONDAY NOVEMBER, 3 9.30AM – 9.45AM

O-4 GENETIC BASIS FOR THE EXTREME SUSCEPTIBILITY OF MBT/PAS MICE TO RIFT VALLEY FEVER VIRUS

Tania Zaverucha Do Valle[1], Agnès Billecocq[2], Robert Geffers[3], Klaus Schughart[4], Michèle Bouloy[2], Xavier Montagutelli[1], Jean-Jacques Panthier[1]
[1]Mouse Functional Genetics Unit, CNRS URA 2578, Institut Pasteur, Paris, France, [2]Molecular Genetics of Bunyaviruses, Institut Pasteur, Paris, France, [3]Array Facility/Cell Biology, Helmholtz Centre for Infection Research, Braunschweig, Germany, [4]Experimental Mouse Genetics, Helmholtz Centre for Infection Research, Braunschweig, Germany

Rift Valley Fever (RVF) is an mosquito-borne viral disease of ruminants and humans, naturally transmitted by RVF virus (genus *Phlebovirus*, family *Bunyaviridae*). RVF is encountered in an enzootic or epizootic form along the east and south coast of Africa. It also circulates in West Africa, Egypt and was recently introduced in Yemen and Saudi Arabia. It is feared that the disease could spread into northernmost territories of Africa and the Middle East. In humans, RVF virus induces usually uncomplicated acute febrile illness but in some cases, a severe and fatal disease can occur associated with liver necrosis, hemorrhagic fever or encephalitis. Mice are susceptible to RVF. To test for genetic contributions to infection susceptibility, we studied several inbred strains. Indeed, MBT/Pas mice are highly susceptible to infection with virulent RVF virus. To analyze the genetic basis for the extreme susceptibility of MBT/Pas mice, we infected animals with virulent RVF virus ZH548 (isolate from the Egyptian outbreak in 1977) and studied the segregation of survival time in 527 (BALB/cByJxMBT/Pas)F2 mice. Several QTLs controlling the susceptibility to RVF were identified. To gain further insights in the cellular mechanisms responsible for the susceptibility of MBT/Pas mice, we applied transcriptional profiling. Expression profiles of embryonic fibroblasts (MEF) from MBT/Pas and BALB/cByJ mice were interrogated using an Affymetrix chip (Mouse430.2) nine hours following RVF infection. Genes differentially regulated in infected MBT/Pas and BALB/cByJ MEF and mapped to QTL are the primary focus of analysis. The results of the analysis will be presented.

IMMUNITY/INFECTION/EPIGENETICS
ORAL PRESENTATION MONDAY NOVEMBER, 3 9.45AM – 10.00AM

O-5 GENETIC ANALYSIS OF ANTIBODY-INDUCED ARTHRITIS USING C57BL/6J-CHRN^{PWD} CONSOMIC STRAINS

Stefka Petkova, Petko Petkov, Beverly Paigen
The Jackson Laboratory, Bar Harbor, United States

Rheumatoid arthritis is a complex disease that involves participation of both genetic and environmental factors, a complexity that current mouse models do not always mirror. New approaches are urgently needed to elucidate the mechanisms underlying susceptibility to this disease, as well as factors influencing both the initiation and perpetuation of systemic inflammation in distal joints. Using a new genetic tool, a set of C57BL/6J-ChrN^{PWD} chromosomal substitution (consomic) strains in which a single chromosome from the wild-derived PWD/PhJ mouse strain is transferred onto a C57BL/6J genetic background and K/BxN serum-induced arthritis model, we have shown that 1) a 2.2-Mb region on PWD/PhJ Chromosome 6 (Chr 6), containing seven genes, confers resistance to serum-induced arthritis, and 2) modifier genes on other chromosomes interact with the Chr 6 region to change its dominance status. None of these seven genes has to date been implicated in rheumatoid arthritis. Flow cytometric, quantitative PCR, and histopathological analyses of peripheral blood and joint tissues demonstrated a joint-specific inflammation in C57BL/6J mice, but not in the PWD/PhJ and C57BL/6J-Chr6^{PWD} consomic strains, which suggest that both genetic and immune factors are key players of arthritis pathogenesis.

These studies provide opportunity to unravel new gene(s) that inhibit arthritis initiation and/or progression and dissect molecular pathways involved in arthritis pathogenesis. Importantly, it allows the mapping of genes modifying the response to arthritogenic stimulus, a task that is extremely difficult if not impossible in humans.

IMMUNITY/INFECTION/EPIGENETICS
ORAL PRESENTATION MONDAY NOVEMBER, 3 10.00AM – 10.15AM

O-6 SURVIVAL AFTER H5N1 VIRUS INFECTION; IT IS IN YOUR GENES

Jacco Boon[1], Robert Williams[2], Lu Lu[2], Richard Webby[1]
[1]St Jude Children's Research Hospital, Memphis, TN, United States, [2]University of Tennessee Health Science Center, Memphis, TN, United States

Despite the prevalence of H5N1 influenza viruses in global avian populations, the number of human cases is comparatively few. Although viral factors almost certainly play a role in limiting human infection and disease, it is likely that host genetics contribute substantially. Indeed, a better understanding of the role of host factors in the infectious process is an important goal for controlling and treating infectious agents of all types. To investigate host factors in the context of influenza infection we determined the 50% mouse lethal dose (MLD_{50}) of the H5N1 virus (A/Hong Kong/213/03 (HK213)) in sixteen inbred mouse strains. The maximum MLD_{50} differences between the strains were 5-logs for HK213. Disease severity following HK213 infection was associated with differences in replication kinetics and a higher production of pro-inflammatory cytokines. Recombinant inbred BXD strains, derived from DBA/2J (susceptible to infection) and C57BL/6 (resistant to infection) mice were utilized to map survival to 3 quantitative trait loci. Analysis of gene expression of lung tissue from infected and uninfected resistant and susceptible mouse strains identified several candidate genes as host components modulating the infectious process.

COMPARATIVE GENOMICS/COMPUTATIONAL BIOLOGY
ORAL PRESENTATION MONDAY NOVEMBER, 3 1.30PM – 1.45PM

O-7 RECOMBINATION LANDSCAPE AND GENETIC BACKGROUND – EVOLUTIONARY CONSERVATION OF REGIONAL BUT NOT LOCAL RATES

Petko Petkov, Lorin Petros, Rose Madeira, Evelyn Sargent, Timothy Billings, Kenneth Paigen
The Jackson Laboratory, Bar Harbor, United States

In most eukaryotic organisms, recombination - the exchange of genetic information between homologous chromosomes - ensures the proper recognition and segregation of chromosomes during meiosis. Recombination events in mammals are not randomly positioned along the chromosomes but occur in preferential 1–2-kilobase sequences termed hotspots. To address the question of how genetic background influences recombination landscape, we have studied recombination rates in two mouse crosses, C57BL/6J x CAST/EiJ (B6xCAST) and WSB/EiJ x PWD/PhJ (WSBxPWD), and prepared detailed sex-specific maps of Chr1 and Chr 11.
The positioning and activity of recombination hotspots are regulated on at least three levels – chromosome-wide, regional and local; each of these levels show sex specificity. The overall chromosomal rates are generally higher in females than in males due to shorter interference distances. Regional recombination rates are dependent on the positioning relative to the centromeres and the telomeres. Significant differences in regional recombination rates may account for a reversal of the effect of the interference on overall recombination rates.
The comparison of recombination rates between the two crosses shows positive correlation on a megabase-scale level. However, the correlation diminishes with distance, and approaches zero at distances below 50 kb. It is apparent that the two crosses use almost entirely different sets of hotspots. However, in each cross females and males share significant proportion of their hotspots, although with different activities. We conclude that genetic background plays an important role in regulation of all aspects of recombination positioning, including sex specificity, regional variation and hotspot placement.

COMPARATIVE GENOMICS/COMPUTATIONAL BIOLOGY
ORAL PRESENTATION MONDAY NOVEMBER, 3 1.45PM – 2.00PM

O-8 TWO WAVE INTERSUBSPECIFIC INTROGRESSION BUILT UP GENOME FRAMEWORK OF THE CLASSICAL LABORATORY MOUSE STRAINS

Toyoyuki Takada[2], Toshinobu Ebata[1], Tadasu Shin-I[1], Takanori Narita[1], Kuniya Abe[3], Yoshiyuki Sakaki[4], Atsushi Toyoda[4], Yuichi Obata[3], Kazuo Moriwaki[3], Yuichi Kohara[1], Toshihiko Shiroishi[1]
[1]National Institute of Genetics, Shizuoka, Japan, [2]Research Organization of Information and Systems, Tokyo, Japan, [3]RIKEN BRC, Ibaraki, Japan, [4]RIKEN GSC, Kanagawa, Japan

Whole genome resequencing of Japanese wild mouse (*Mus musculus molossinus*)-derived inbred strain MSM/Ms and direct comparison of the sequence with C57BL/6J (B6) genome revealed totally 9.7 million high-quality SNPs including 6.4 million novel SNPs. Approximately 6.1% of B6 genome has long sequence segments (Ave. 248kb in length) highly homologous to the MSM/Ms sequence. Comparison of the homologous sequences of B6 with those of many wild-derived strains verified that they represent recent introgression from molossinus genome. Remaining regions of B6 genome also has much shorter sequences highly homologous to either sequence of musculus or molossinus, which are likely vestiges of much older introgression through European hybrid zone. Further comparative analysis of the NIEHS SNP data and ours demonstrated that the two waves introgression into the west European subspecies domesticus built up genome framework of the classical laboratory strains. Both types of introgression of subspecific genomes, which occur in the critical coding and non-coding sequences, should be responsible for phenotypes characteristic to the classical laboratory strains and QTLs for complex traits. It is conceivable that if given QTL is caused by a SNP in the first wave introgression, it would be less difficult to identify the SNP, because total number of candidate SNPs to be examined is relatively small. Thus, it may be possible to predict difficulty to identify a SNP responsible for given QTL, based on information of whether the QTL of interest is confined or linked to the first or second wave introgression.

COMPARATIVE GENOMICS/COMPUTATIONAL BIOLOGY
ORAL PRESENTATION MONDAY NOVEMBER, 3 2.00PM – 2.15PM

O-9 COMBINED GENOTYPE AND EXPRESSION ANALYSIS OF INBRED AND DERIVED CONGENIC MICE SUGGEST THAT MOST GENE EXPRESSION IS INDIRECTLY *TRANS* REGULATED BY MECHANISMS THAT ARE SENSITIVE TO GENETIC BACKGROUND

Harry Noyes[1], Morris Agaba[2], SunJong Oh[3], Susan Anderson[4], Alan Archibald[4], Andy Brass[5], John Gibson[5], Laurence Hall[4], Helen Hulme[5], Steve Kemp[1]
[1]University of Liverpool, Liverpool, United Kingdom, [2]International Livestock Research Institute (IRLI), Nairobi, Kenya, [3]National Institute of Animal Institute, RDA, Suwan, Korea, Democratic People's Republic of, [4]Roslin Institute, Roslin, United Kingdom, [5]University of Manchester, Manchester, United Kingdom, [6]University of New England, Armidale, Australia

Inbred laboratory strains of mice are among the primary tools for investigating the relationship between gene sequence, gene expression and phenotype. Whilst it has been recognised for a many years that the effect of a gene or more correctly an allele or mutation may be modified depending upon genetic background, it has been difficult to quantify the impact of such genetic background effects. To address this, we have combined a public murine 8 million SNP data set with Affymetrix expression data from inbred mice and sets of congenic mice derived from them under twelve conditions. Analysis of these data has provided estimates of the proportions of genes that show *cis* and *trans* regulation. Thirty-six (36%) of differentially expressed genes appeared to be *cis* regulated and showed evidence of consistent and tissue-independent expression. In contrast, the expression of the remaining *trans* regulated genes appeared to be sensitive to the genetic background. Analysis of gene expression in congenic lines, in which small regions of the genome from one inbred line are introduced onto a background of another line, showed that the genes that were differentially expressed between congenics and their matched controls were not a subset of those that were differentially expressed between the parents, showing that these were also dependent on genetic background. The evidence that most gene regulation is strongly influenced by genetic background, suggests that pathways that are modified by an allelic variant, may not show expected differential expression except in the specific genetic backgrounds in which they were identified. This amounts to a large discovery bias in the identification of key genes by typical mapping approaches.

COMPARATIVE GENOMICS/COMPUTATIONAL BIOLOGY
ORAL PRESENTATION MONDAY NOVEMBER, 3 2.15PM – 2.30PM

O-10 NANOCAGE PROMOTOME ANALYSIS OF THE MOUSE OLFACTORY EPITHELIUM.

Charles Plessy[1], Nicholas Bertain[1], Roberto Simone[2], Giovanni Pascarella[2], Cristina Vlachouli[2], Signe Olivarious[1], Dean Lazarevich[2], Claudia Carrieri[2], Altuna Akalin[3], Boris Lenhard[3], Stefano Gustincich[2], Piero Carninci[1]
[1]RIKEN Omics Science Center, Yokohama, Japan, [2]The Giovanni Armenise-Harvard Foundation Laboratory, International School for Advanced Studies (SISSA), Trieste, Italy, [3]Bergen Center for Computational Science, University of Bergen, Bergen, Norway

Sequencing-based methods for gene regulation analysis become affordable thanks to breakthroughs in technology. However, the preparation of the samples to be sequenced still requires high quantities of starting materials and skilled personnel, hampering its application to microdissected material. We developed nanoCAGE, based on Cap Analysis Gene Expression, as a simple method to analyse the gene network of small samples and requires only some 50 ng of total RNA (few thousands cells). Because it uses random priming and only captures the 5' end of capped molecules, nanoCAGE is a technique of choice when the RNA quality and/or quantity is not optimal, such as with RNA extracted from histological slices by laser-capture microdissection (LCM), flow-sorted cells, biopsies, etc.
We microdissected the olfactory epithelium of adult and juvenile mice, and analysed its promotome with nanoCAGE. Most olfactory receptors (OR) are only known by their predicted coding sequences. Using libraries totaling more than 30,000,000 CAGE tags, we determined the transcription start sites of the OR genes and systematically identified their conserved upstream sequences. We report the comparative phylogenetical analysis of the conserved promoters and coding sequences whose expression they control. We also detected antisense transcription in the OR gene clusters and confirmed the presence of these new non-coding RNAs by RACE-PCR and in situ hybridisation. Using nanoCAGE promoters, sense/antisense pairs and prediction of the binding sites of the transcription factors whose expression is detected by nanoCAGE, we computed a regulatory network that characterises the transcriptome of the olfactory epithelium.

COMPARATIVE GENOMICS/COMPUTATIONAL BIOLOGY
ORAL PRESENTATION MONDAY NOVEMBER, 3 2.30PM – 2.45PM

O-11 GENETIC STRUCTURE OF THE PRE-COLLABORATIVE CROSS (PRECC) POPULATION

David Aylor[1], David Threadgill[1], Leonard McMillan[1], Gary Churchill[2], Elissa Chesler[3], Fernando Pardo-Manuel de Villena[1]
[1]University of North Carolina, Chapel Hill, NC, United States, [2]The Jackson Laboratory, Bar Harbor, ME, United States, [3]Oak Ridge National Laboratory, Oak Ridge, TN, United States

The Collaborative Cross (CC) is being developed as an ideal resource for mammalian system genetics. The population will harbour maximal genetic variation with ample recombination for high-resolution mapping. The PreCC is a proof-of-concept experiment that provides an empirical assessment of the CC's genetic properties. We report the genetic structure of 200 distinct CC lines that have undergone at least six generations of inbreeding. The PreCC provides an opportunity to measure both diversity and recombination. The eight founder strains were selected for genetic diversity, and we assess the amount conserved in each individual inbred line and in the population. We estimate the contribution of each parent to each of the 200 lines. While each line is a mosaic of all eight parents, stochastic processes lead to variation in parental contributions. Nonetheless, the founder haplotypes should be equally represented in the population due to the high number of lines. Diversity provides the basis for variation in complex traits, but the ability to map traits depends on the distribution and frequency of recombination within a population. We assess the resolution of recombination breakpoints, the average distance between breakpoints, and the variance of this distance. We compare these measures with theoretical estimates. This snapshot of diversity and recombination within this cohort provides direct and strong evidence of the power of the Collaborative Cross for complex trait analysis.

COMPARATIVE GENOMICS/COMPUTATIONAL BIOLOGY
ORAL PRESENTATION MONDAY NOVEMBER, 3 2.45PM – 3.00PM

O-12 INVESTIGATION OF HIPPOCAMPAL MICRORNA EXPRESSION AND FUNCTION IN C57BL/6J X DBA/2J RECOMBINANT INBRED MICE (BXD RI)

Michael Parsons[1], Christina Grimm[3], Jose Paya-Cano[4], Cathy Fernandes[2], Lin Liu[2], Wilfried Nietfeld[3], Hans Lehrach[3], Leonard Schalkwyk[2]
[1]MRC Mammalian Genetics Unit, Harwell, Didcot, United Kingdom, [2]Social, Genetic, and Developmental Psychiatry Research Centre, Institute of Psychiatry, KCL, London, United Kingdom, [3]Max-Planck-Institute for Molecular Genetics (MPIMG), Berlin, Germany, [4]Wolfson Centre for Age Related Diseases, Hodgkin Building, London, United Kingdom

Micro-RNAs (miRNAs) are short single-stranded non-coding RNAs that are involved in the regulation of gene expression. miRNAs have been shown to be involved in the regulation of numerous biological processes as well as being implicated in various diseases. In a previous study, we detected 5 miRNAs with differences in hippocampal expression between the C57BL/6J and DBA/2J strains: *miR-15b, miR-31, miR-34c, miR-212, miR-301a* ($p<0.05$). In order to further characterise the effects of these miRNAs, we conducted Taqman RT-PCR assays for these miRNAs (plus 3 control assays) using hippocampal RNA taken from 24 C57BL/6J x DBA/2J recombinant inbred mouse strains (BXD RI) and the two parental strains (n=4). These BXD RI mice had previously been behaviourally characterised using an extensive behavioural battery including measures of anxiety, activity, exploration and cognitive ability. We correlated the expression of these miRNAs with *in silico* phenotype data, mRNA expression and our in house behavioural measures in order to determine the effects of these miRNAs on both phenotype and the regulation of mRNA expression. We found that *miR-15b* is correlated with various memory related measures, while both *miR-212* and *miR-301a* are both correlated with numerous exploration measures. Furthermore, we conducted eQTL analysis using WebQTL (www.genenetwork.org) to determine the genetic loci that affect the expression of these miRNAs, resulting in six QTL peaks above suggestive significance. Together these approaches have allowed us to gain a better in sight into the function of these miRNAs and to nominate candidate miRNAs for various behavioural traits including memory and exploration.

MODELING DISEASE
ORAL PRESENTATION TUESDAY NOVEMBER, 4 9.00AM – 9.15AM

O-13 AN ENU-INDUCED MUTATION OF A MIRNA ASSOCIATED WITH PROGRESSIVE HEARING LOSS

Morag Lewis[1], Elizabeth Quint[2], Anne Glazier[1], Helmut Fuchs[3], Martin Hrabe de Angelis[3], Cordelia Langford[1], Stijn van Dongen[1], Cei Abreu-Goodger[1], Nick Redshaw[4], Tamas Dalmay[4], Miguel Angel Moreno Pelayo[5], Anton Enright[1], Karen Steel[1]
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Progressive hearing loss is very common in the human population, but relatively little is known about the molecular or genetic basis. We have described a new ENU-induced mouse mutant, diminuendo (*Dmdo*), which has a single base change in the seed region of microRNA miR-96. Heterozygotes show progressive loss of auditory responses and sensory hair cell stereocilia bundle anomalies, while homozygotes have no cochlear responses and extensive hair cell loss by four weeks old. We have shown miR-96 is expressed in both mutant and wildtype hair cells. MicroRNAs downregulate target genes by binding to their mRNAs at specific sites, so a seed region mutation would be expected to lead to upregulation of target genes. Using bioinformatic analysis, we predicted potential miR-96 targets and confirmed five by luciferase assay, but immunolabelling and QRTPCR suggested little or no upregulation of these genes in homozygote hair cells. Microarray analysis revealed 96 genes with significantly altered expression in mutant tissues, including several important for hair cell development and function. Hypergeometric analysis showed that among the upregulated genes, the two heptamers complementary to the miR-96 seed were significantly enriched, while the heptamers complementary to the mutant seed were enriched in the downregulated genes, indicating that the mutation causes not only a loss of wildtype targets but also a gain of novel targets. This is the first microRNA found to be associated with deafness, and represents a model for understanding and potentially influencing the progression of hair cell dysfunction and degeneration in progressive hearing loss more generally.

MODELING DISEASE
ORAL PRESENTATION TUESDAY NOVEMBER, 4 9.15AM – 9.30AM

O-14 GENERATION AND ANALYSIS OF NOVEL ENU-INDUCED MOUSE MODELS FOR HUMAN NEPHROPATHIES

Birgit Rathkolb[1], Elisabeth Kemter[1], Anja Schrewe[2], Wolfgang Hans[3], Jan Rozman[4], Christina Schessl[1], Corinna Moerth[1], Matthias Klaften[3], Sibylle Wagner[3], Kateryna Butuzova[3], Helmut Fuchs[3], Valerie Gailus-Durner[3], Boris Ivandic[2], Martin Hrabé de Angelis[3], Martin Klingenspor[4], Ruediger Wanke[5], Eckhard Wolf[1], Bernhard Aigner[1]
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Kidney diseases lead to the failure of urinary excretion of metabolism products. In the Munich ethylnitrosourea (ENU) mouse mutagenesis project, which is done on the C3H inbred genetic background, G1 and G3 offspring of ENU-treated mice were screened for increased plasma urea levels to develop novel models for human nephropathies. Seven mutant lines harbouring a dominant or recessive mutation leading to increased plasma urea levels were established.
Pathologic investigations of kidneys from these mouse lines revealed a broad spectrum of macroscopic and histologic alterations. Linkage analysis of the causative mutation and subsequent candidate gene analysis identified the mutation in two of the nephropathy mouse lines and resulted in new mutant alleles of solute carrier family 12 member 1 (Slc12a1) and uromodulin (Umod). These lines were phenotypically analyzed using standardized screens in the German Mouse Clinic (GMC). In both lines, similar changes in clinical-chemical plasma and urine parameters, bone mineralization, energy metabolism and plasma levels of atrial natriuretic peptide were detected in mutant mice. In addition decreased blood pressure in homozygous Slc12a1 mutants and decreased body temperature in Umod mutant mice were shown. Thus, novel ENU-induced mutant mouse lines were established which may serve as models for human kidney diseases that are linked to mutations in the same genes. Beside that these results demonstrate the efficiency of ENU mutagenesis to create mouse models of human diseases and the systemic phenotypic analysis of mutant mouse lines in the GMC.

MODELING DISEASE
ORAL PRESENTATION TUESDAY NOVEMBER, 4 9.30AM – 9.45AM

O-15 CARDIAC TROPONIN I KINASE MODIFIES DISEASE PROGRESSION AND OUTCOME IN MOUSE MODELS OF CARDIOMYOPATHY

Ferrin Wheeler, Odessa Marks, Tracy Hadnott, Pei-Lun Chu, Lan Mao, Howard Rockman, Douglas Marchuk
Duke University, Durham, North Carolina, United States

The Calsequestrin (CSQ) mouse model of cardiomyopathy exhibits variation in the phenotype progression and severity highly dependent on the genetic background. QTL mapping in the context of the transgenic sensitizer has yielded seven heart failure modifier (*Hrtfm*) loci which modify disease progression. One locus, *Hrtfm2*, was mapped in crosses with different inbred strains, enabling shared haplotype analysis to narrow the candidate interval. Here we report that a single gene in the interval, *Tnni3k* (cardiac troponin I kinase), shows a significant difference in transcript levels between strains. Strains with a susceptible phenotype (C57/BL6, AKR) show high levels of transcript while strains that express low transcript levels (DBA/2J) are protected. In strains showing reduced message levels, we have identified an intronic SNP that activates a cryptic splice site leading to aberrant splicing, followed by nonsense-mediated decay of the message and an absence of detectable protein. As predicted by its disease-modifying role, transgenic animals over-expressing human TNNI3K exhibit no cardiac phenotype in isolation. However, when crossed with the CSQ transgenic sensitizer, double transgenic animals exhibit impaired systolic function and drastically reduced survival, indicating that TNNI3K modifies disease progression in the CSQ model. In contrast, transgenic animals expressing a kinase dead version of the transgene have no effect on the phenotype. We further show that expression of active TNNI3K also modifies disease progression in a pressure-overload surgical model of heart disease. These combined data provide a new gene for investigation in the genetics of heart disease, and provide a novel target for therapeutic intervention.

MODELING DISEASE
ORAL PRESENTATION TUESDAY NOVEMBER, 4 9.45AM – 10.00AM

O-16 THE *BARTHEZ* MOUSE MUTATION IS A HYPOMORPHIC ALLELE OF THE *ASS1* GENE.

Fernando Benavides[1], Jean Jaubert[3], Carlos Perez[1], Carlos Quintanilla[1], Isabelle Aubin[3], Kirstin Barnhart[2], Irma Gimenez-Conti[1], Jean-Louis Guenet[3], Donna Kusewitt[1], Claudio Conti[1]
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Here we present *barthez* (*bar*), a new autosomal recessive mutation with a complex phenotype. Homozygous *bar/bar* mice are easily recognized a few days after birth due to severe retardation in post-natal development, alopecia with scaly skin, ataxia and circling behavior. Numerous pathological abnormalities become evident within two weeks of parturition. The most notable findings include delayed cerebellar development, epidermal hyperkeratosis and follicular dystrophy.

Using F2 mice we mapped the *bar* locus to chromosome 2, between markers D2Mit153 and D2Mit367 (29.2 - 33.4 Mb), a region homologous with human chromosome 9q34.1. During the sequencing of candidate genes in this region, a mutation was detected in exon 12 of the *Ass1* (argininosuccinate synthetase 1) gene. This cytosolic enzyme catalyzes the third step in the urea cycle, in which citrulline is condensed with aspartate to form argininosuccinic acid. The *barthez* mutation is a C-T transition that results in an aminoacid change from Arg to Cys in residue 265 (R265C). In order to determine if this putative hypomorphic mutation expresses reduced levels of *Ass1* RNA transcripts, we analyzed total liver RNA by quantitative real-time PCR. No significant differences were detected between affected mutants and control littermates.

The disease condition exhibited by homozygous *bar/bar* mice is analogous to human Citrullinemia type I (CTLN1, OMIM# 215700), a rare inherited disorder caused by deficiency of the ASS1 enzyme. In fact, the C-T transition in these mutants parallels a clinical mutation that has been described previously in human patients affected by a mild phenotype of CTLN1. In our animal model, homozygous *bar/bar* mice show a 40-fold increase in plasma citrulline and a 2- to 4-fold increase in the plasma levels of almost all amino acids. Liver ASS activity is severely reduced and plasma ammonia concentration is elevated (>1000 µg/dl) in mutant mice. By reducing the blood ammonia levels with the administration of sodium benzoate and arginine we were able to significantly increase the survival rate of the mutant mice.

Infants with the acute neonatal form of CTLN1, one of the urea cycle disorders, appear normal at birth but develop hyperammonemia and become progressively lethargic and may experience signs of increased intracranial pressure, including spasticity, seizures, loss of consciousness, and death. Children who are treated promptly may survive for some time, but usually with significant neurologic deficits. Considering that *Ass1* knock-out mice die shortly after birth, hypomorphic $Ass1^{bar}/Ass1^{bar}$ mice may constitute an interesting model for mechanistic and preclinical studies of CTLN1 that will allow the development of more aggressive and effective therapies.

MODELING DISEASE
ORAL PRESENTATION TUESDAY NOVEMBER, 4 10.00AM – 10.15AM

O-17 THE PRECC, A COLLABORATIVE AND INTERDISCIPLINARY EXPERIMENT BASED ON EMERGING RI LINES

Fernando Pardo-Manuel de Villena[1], Samie Ahmed[1], David Aylor[1], Ralph Baric[1], Timothy A. Bell[1], Lisa Branstetter[2], Elissa Chesler[2], Yvette Chuang[1], Gary Churchill[3], Francis S Collins[4], Wendy Foulds Mathes[1], Mark Heise[1], Kunjie Hua[1], Andrew Hulbert[1], Fuad Iraqi[5], Samir Kelada[4], Ron Korstanje[3], Kari Kubalanza[4], Kenneth Manly[6], Leonard McMillan[1], Darla Miller[2], Grant Morahan[7], Richard Mott[8], Derrick Nehrenberg[1], Daniel Pomp[1], Christine Powell[1], David A. Schwartz[9], Chris Sproul[1], Jill Steigerwalt[1], David Threadgill[1], William Valdar[8], Wei Wang[1], Michelle Weed[1], Alan Whitmore[1], Rob Williams[10], Yuying Xie[1], Binnaz Yalcin[8], Marc Zylka[1]
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The Collaborative Cross (CC) is a large panel of recombinant inbred (RI) lines specifically designed to support research on systems genetics and predictive biology. Three separate populations (ORNL, Wellcome Trust/TAU and Perth/Morahan) have been derived from a common set of eight founder inbred strains. The founder strains were selected based on reproductive performance, genetic diversity and existing genomic reagents. Here we describe an initial genetic study that incorporates the main themes behind the design of the CC: complex biological traits, multi-investigator/institution collaborations, state of the art phenotyping and genomic analysis, and interdisciplinary data integration. Males from ORNL and Wellcome Trust/TAU lines that have reached at least the G2:F6 generation of inbreeding (i.e. the preCC) will be analyzed for body weight, kidney function and lipid metabolism (Churchill/Iraqi/Mott/Yalcin). ORNL lines are undergoing a phenotyping battery at UNC centered on the effect of voluntary exercise on energy intake, metabolic rate, body mass and composition, and bone (Foulds Mathes/Pomp). We are also testing cardiac function, alcohol metabolism (Powell/Threadgill), pain perception (Chuang/Zylka), aggression (Nehrenberg/Pomp), reproductive capacity (Ahmed/Bell), and establishing a tissue collection at UNC. Furthermore, siblings of these males are being tested for asthma susceptibility (Kelada/Collins) and for susceptibility to the viral pathogens SARS and influenza (Baric/Heise/Ferris). Finally, all lines will be genotyped at high density using custom designed 300K SNP Affymetrix microarrays. Analytical and visualization tools and dedicated databases are being developed for this experiment (McMillan/Wang). Abstracts describing specific details of the phenotyping pipelines and genetic architecture of the preCC mice have been submitted separately.

MODELING DISEASE
ORAL PRESENTATION TUESDAY NOVEMBER, 4 10.15AM – 10.30AM

O-18 DETECTING LOCI THAT CONFER SUSCEPTIBILITY TO DUST-MITE INDUCED ASTHMA USING A PANEL OF PreCC MICE

Samir Kelada[1], Kari Kubalanza[1], Greg Whitehead[2], Elissa Chesler[3], Darla Miller[3], Ken Manly[4], Gary Churchill[5], Fernando Pardo-Manuel de Villena[6], David Schwartz[7], Francis Collins[1]
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We aim to identify genes that confer susceptibility to allergic asthma, a disease of considerable public health importance. To date, we have established a mouse model using house dust mite (HDM) allergen that produces key features of asthma, namely airway hyper-responsiveness, inflammation, and mucus production. Importantly, we observed that the effects of HDM exposure are clearly strain-dependent, indicating a genetic component to HDM response. To identify susceptibility loci, we are using a newly established population of genetically diverse mice, the PreCC, that are derived from an eight-way cross of common (A/J, C57BL/6J, 129/SvImJ, NOD/ShILtJ and NZO/HILtJ) and wild-derived (WSB/EiJ, PWK/PhJ and CAST/EiJ) inbred strains known as the Collaborative Cross (CC). The CC was designed to capture maximal genetic diversity of inbred strains and at the same time minimize the effects of population structure, thereby overcoming many of the limitations of previous mapping approaches. Here we show that a population of PreCC mice bred at Oak Ridge National Laboratory varies dramatically in baseline lung function and in response to HDM, and that these phenotypes are correlated. Additionally, we observed that the degree of airway inflammation does not correlate with impaired lung function. Once the entire panel of PreCC mice have been phenotyped, loci will be identified by QTL mapping, and the results will be used to guide candidate gene selection in a case-control study of asthma susceptibility in humans.

DEVELOPMENT/CANCER BIOLOGY/EVOLUTION AGING
ORAL PRESENTATION TUESDAY NOVEMBER, 4 1.30PM – 1.45PM

O-19 AN ALLELE OF *PLZF* REVEALS FUNCTIONAL DOMAINS WITH DISTINCT ROLES IN SKELETAL PATTERNING AND SPERMATOGONIAL STEM CELL RENEWAL

Yung-Hao Ching[1], Lawriston Wilson[2], John Schimenti[3]
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The promyelocytic leukemia zinc finger gene *Plzf* (also called *Zbtb16, Zfp145* or *Green's luxoid*) belongs to the POZ/zinc-finger family of transcription factors. It contains a BTB/POZ domain that mediates epigenetic transcriptional repression. PLZF is essential for proper skeleton patterning and male germ cell renewal. Two alleles have been reported that display similar phenotypes: a targeted knock-out, and the spontaneous nonsense mutation *luxoid*. We describe a new ENU induced missense allele of *Plzf* called seven toes ($Plzf^{7t}$). Homozygous animals exhibit hindlimb and axial skeleton abnormalities. Whereas the skeletal abnormalities are similar to those of the other alleles, $Plzf^{7t}$ differs in that it does not cause spermatogonial depletion phenotype. Positional cloning revealed a point mutation resulting in a Glu44Gly alteration of an evolutionally conserved amino acid, which is predicted to altered the BTB domain of the protein. Therefore, $Plzf^{7t}$ is a separation-of-function allele that compartmentalizes the distinct functions of PLZF. The data indicate that the N terminus of the protein is important for limb and skeletal patterning, whereas the C terminus is involved in the spermatogonial renewal function.

DEVELOPMENT/CANCER BIOLOGY/EVOLUTION AGING
ORAL PRESENTATION TUESDAY NOVEMBER, 4 1.45PM – 2.00PM

O-20 GENETIC ANALYSIS OF AGING IN 31 INBRED STRAINS OF MICE AND THE IMPORTANCE OF THE IGF-1 PATHWAY

Rong Yuan, Shirng-Wern Tsaih, Shuqin Xing, Cheryl Ackert-Bicknell, Molly Bogue, Luanne Peters, Beverly Paigen
The Jackson Laboratory, Aging Center, Bar Harbor, ME, United States

To better characterize aging in mice, the Jackson Aging Center carried out a lifespan study of 31 genetically-diverse inbred mouse strains housed in a specific pathogen-free facility. We evaluated lifespans of 96 mice/strain, carrying out clinical assessments every 6 months for multiple aging-related phenotypes, including neuromuscular, kidney and heart function, body composition, bone density, hematology and immune system parameters, apoptosis, DNA repair and chromosome fragility, hormonal levels, and histopathology. As data from this study become available, it will be deposited in the Mouse Phenome Database (MPD; www.jax.org/phenome).

In this report we describe the study design, survival curves, median lifespans, and IGF-1 levels. Survival curves varied considerably among strains. Median lifespans ranged from 251-901 days, and median lifespans of males and females from the same strains were significantly correlated (0.77, $P < 0.001$). Plasma levels of IGF-1 at 6 months were negatively correlated with median lifespan. A genome-wide association of median lifespan with haplotype revealed 12 suggestive lifespan quantitative trait loci (QTLs), some of which co-localized with lifespan QTLs previously determined in F2-mapping crosses. Many of these QTL co-localized with IGF1 QTL or genes in the IGF1 pathway. This characterization of aging in a wide range of inbred strains will facilitate research into the genetics of aging.

DEVELOPMENT/CANCER BIOLOGY/EVOLUTION AGING
ORAL PRESENTATION TUESDAY NOVEMBER, 4 2.00PM – 2.15PM

O-21 *IFI207*, A NOVEL IFI200 FAMILY MEMBER SUPPRESSING CELL GROWTH AND PROLIFERATION, IS A CANDIDATE FOR THE PLASMACYTOMA MODIFIER OF RESISTANCE/SUSCEPTIBILITY (*PCTMR*) ON MOUSE CHROMOSOME 1

Ke Zhang, Daniel Kagan, Wendy DuBois, Valery Bliskovsky, William C. Vass, Beverly A. Mock
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Murine Ifi200 genes are homologous with the human 200 amino acid repeat (HIN-200) family of hematopoietic interferon-inducible nuclear antigens. They are implicated in regulating cell growth/cell cycle progression via interactions with tumor suppressors, Rb and p53. We identified a novel murine Ifi gene, *Ifi207*, located in the IFI/HIN-200 cluster on mouse Chr 1, near *Fcgr2*, during a screen for differentially expressed genes in granuloma tissues derived from the mesenteries of pristane-primed BALB/c and DBA mice. Expression of the BALB/c allele was > 500-fold higher than the DBA allele. At least 4 genes determine susceptibility/resistance patterns in backcross mice between these two strains. In an earlier genome wide association study (PNAS 90: 9499), tumor susceptibility was associated with BALB/c alleles of Chr 4 genes and heterozygosity (C/D) of Chr 1 genes. We have designated this Chr 1 locus as a plasmacytoma modifier of resistance, *Pctmr*; it was the only locus where susceptibility was linked to the DBA allele. Genomic sequence analysis of BALB/c and DBA clones revealed a genetic polymorphism of *Ifi207* between these two mouse strains. Overexpression of the BALB/c *Ifi207* gene in murine cell lines suppresses growth. This biological activity, together with the disease susceptibility of heterozygotes at the *Pctmr* locus, suggest that *Ifi207* is likely to be a tumor suppressor gene that displays haplo-insufficiency. This novel gene also becomes a candidate to examine in other disease phenotypes mapping within this cluster of genes on mouse Chr 1.

DEVELOPMENT/CANCER BIOLOGY/EVOLUTION AGING
ORAL PRESENTATION TUESDAY NOVEMBER, 4 2.15PM – 2.30PM

O-22 THE DEVELOPMENTAL EFFECTS OF FBOX11

Hilda Tateossian, Rachel Hardisty-Hughes, Susan Morse, Maria R. Romero, Helen Hilton, Charlotte Dean, Steve Brown
Medical Research Council, Harwell, United Kingdom

The ENU induced mouse mutant *Jeff* was previously described as having chronic proliferative otitis media. The *Jeff* mutation was cloned and found to be *Fbxo11*, a member of the F-box family (Hardisty et al. 2006, *Hum. Mol. Genet.* 15: 3273). Fbox proteins function as part of an SCF (SKP1-cullin-F-box) protein ligase complex, recognizing and binding phosphorylated proteins and promoting their ubiquitination and degradation. The role of *Fbxo11* during mouse development remains unclear. Mice homozygous for *Jeff* are born with cleft palate, eyelids open; they have impairment of respiratory function and die within a few hours of birth. This study aims to investigate the palate, eyelid and lung phenotypes in the *Jeff* homozygous mice in order to uncover the underlying pathways involved with *Fbxo11* function. We used a combination of immunohistochemistry to assess protein localisation of salient members of the TGF-□ family signalling pathway (TGF-□3, TGF□ RI, Smad2, Smad3 and Smad4) and generation of compound mutants (using Smad2 and p53 KO mice) to assess genetic interactions. Our observations support a model whereby in palate, eyelid and lung, Fbxo11 dependent modification is required to limit the accumulation of phospho-Smad2 in the nucleus of epithelial cells of palatal shelves, eyelids and airways of the lungs. Mice heterozygous for both *Jeff* and Smad2 mutations recapitulate the *Jeff* homozygote phenotype in the palate and lungs, suggesting they are direct or indirect interacting partners in the development of these two tissues.

DEVELOPMENT/CANCER BIOLOGY/EVOLUTION AGING
ORAL PRESENTATION TUESDAY NOVEMBER, 4 2.30PM – 2.45PM

O-23 CORRELATION STUDIES OF MICRORNA EXPRESSION, THEIR PUTATIVE DOWNSTREAM TARGETS AND EQTLS IN HEMATOPOIETIC DEVELOPMENT

Leonid Bystrykh, Thomas de Jong, Olya Kalmykowa, Alice Gerrits, Gerald de Haan
UMCG, Groningen, Netherlands

Regulation of gene expression is a complex event controlled by various loci in the genome usually named expression Quantitative Trait Loci (eQTL). Using mouse recombinant inbred mice we purified hematopoietic stem cells, progenitors, granulocyte and erythrocyte precursors and performed expression analysis with Illumina micro arrays (www.genenetwork.org). Some genes are known to be physically linked to the expression of exonic or intronic micro RNAs. This allowed us to study both regulation of gene expressions and possible regulatory effects of known mouse microRNAs (miRs) (http://microrna.sanger.ac.uk/sequences/). We selected all genes which could be potential targets of a particular miR and assessed whether these putative targets would be regulated by the genetic locus where the miR resides.
The list of the genes which are both regulated from the QTLs where corresponding miR resides as well as possessing predicted binding site of the same miR appeared to contain about 6200 genes per 208 miRs, residing in 135 genomic clusters. A total distribution of correlations between all genes associated with expression of miRs and their potential targets did not show any skewing to negative values (which is expected from negative effects of miRs to their downstream targets). It indicates either too relaxed prediction algorithm for downstream targets or overestimation of possible effects of each microRNA on its potential targets at natural levels of microRNA expression. Newertheless, we revealed several interesting miRs whose expression could be specifically related to one of a studied hematopoietic cell lineages. These microRNA could be potentially involved in hematopoietic differentiation. To verify candidate downstream targets we are currently developing a retroviral vector wich will allow intronic coexpression of miRs together with reporter and other genes of interest. More complex interaction models of miR-gene expression will be discussed.

OTHER GENOMES AND NEW APPROACHES
ORAL PRESENTATION TUESDAY NOVEMBER, 4 3.30PM – 3.45PM

O-24 KURMA: AN ENU-INDUCED MUTANT ARCHIVE FOR GENE-TARGETING IN RATS

Tomoji Mashimo, Birger Voigt, Akiko Takizawa, Takashi Kuramoto, Tadao Serikawa
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Recently, we reported the combination of ENU mutagenesis with a high-throughput screening assay using the Mu-transposition reaction (MuT-POWER) and intracytoplasmic sperm injection (ICSI) for the recovery of the rare heterozygous genotypes from a frozen sperm repository, which provides numbers of mutations in the rat (Mashimo T *et al*, Nature Genetics 2008). Using the characteristic of the Mu transposition reaction that shows a strong target site preference for single-nucleotide mismatches, MuT-POWER technology proved to be a very powerful tool to detect point mutations in ENU mutagenized G1 animals. The Kyoto University Rat Mutant Archive (KURMA), now comprises of 5,000 G1 male samples that are preserved at the NBRP-Rat and is going to be expanded to 10,000 G1 animals in the near future, which will increase the possibility of finding mutations of a wider variety and in further genes. This large repository of ENU-induced mutations would allow the production of several rat models for human diseases, including hypertension, diabetes, cancer or epilepsy. The mutations are already there – they just need to be found (screened).

OTHER GENOMES AND NEW APPROACHES
ORAL PRESENTATION TUESDAY NOVEMBER, 4 3.45PM – 4.00PM

O-25 THE BXH/HXB RAT RECOMBINANT INBRED STRAINS FOR GENETIC ANALYSES OF THE METABOLIC SYNDROME

Dr. Michal Pravenec[1], Dr. Vaclav Zidek[1], Dr. Petr Mlejnek[1], Dr. Alena Musilova[1], Dr. Ludmila Kazdova[2], Dr. Enrico Petretto[3], Dr. Stuart Cook[3], Prof. Vladimir Kren[4], Prof. Norbert Hubner[5], Dr. Daniel T. O`Connor[6], Prof. Timothy J. Aitman[3], Prof. Theodore W. Kurtz[7]

[1]Institute of Physiology, Czech Academy of Sciences, Prague; [2]Institute for Clinical and Experimental Medicine, Prague; [3]Imperial College London; [4]First Medical Faculty, Charles University, Prague; [5]Max Delbruck Center for Molecular Medicine, Berlin; [6]University of California, San Diego; [7]University of California, San Francisco

The BXH/HXB sets (N=30) of recombinant inbred (RI) strains were derived from reciprocal crosses of BN-*Lx*/Cub and SHR/Ola progenitors that are only distantly related and differ in mutliple cardiovascular and metabolic traits. All RI strains are beyond F65 generation. The most important advantages of the BXH/HXB RI strains for analyses of complex traits are (1) cumulativeness of all results, including over 13,000 SNPs that bin into approximately 1,200 SDPs, in addition, information on copy number variation, over 200 physiological, mainly metabolic, cardiovascular, and biochemical traits (www.genenetwork.org;), as well as gene expression profiles determined with Affymetrix expression arrays in tissues relevant to the pathogenesis of metabolic syndrome (www.genenetwork.org, web. bioinformatics.ic.ac.uk/eqtlexplorer), (2) the availability of complete genome sequences of both progenitor strains. Recently, the BXH/HXB RI strains have been used for the identification at the molecular level of quantitative trait loci (QTLs) associated with complex cardiovascular and metabolic traits, including *Cd36* deletion variant associated with insulin resistance, dyslipidemia as well as with hypertension (Aitman et al., Nat. Genet. 21: 1999; Pravenec et al., Nat. Genet. 27: 2001; Pravenec et al., Nat. Genet. 40: 2008), mutated *Srebf1* associated with hepatic cholesterol levels (Pravenec et al., Hypertension 51:, 2008), *Ogn* underlying cardiac mass (Petretto et al., Nat. Genet. 40: 2008) and *Pnmt* and *Dbh* associated with the regulation of catecholamine synthesis, storage and secretion. These studies demonstrate that the BXH/HXB RI strains represent an efficient model system for analyses of the metabolic syndrome and for identification of QTLs at the molecular level.

OTHER GENOMES AND NEW APPROACHES
ORAL PRESENTATION TUESDAY NOVEMBER, 4 4.00PM – 4.15PM

O-27 MUTATION DISCOVERY IN THE MOUSE USING GENETICALLY GUIDED ARRAY CAPTURE AND RE-SEQUENCING

Mark D'Ascenzo[1], Carl Meecham[2], Jacob Kitzman[1], Christina Middle[1], Todd Richmond[1], Thomas J. Albert[1], Jim Knight[2], Roger Winer[2], Jason Affourtit[2], Jeffrey A. Jeddeloh[1], Leah Rae Donahue[3] and Laura G. Reinholdt[3]
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The Knockout Mouse Project (KOMP) and the various gene trap consortia represent the pinnacle of reverse genetics in the mouse, as they are rapidly advancing the goal of creating a null allele of every gene in the mouse genome. However, allelic series like the Kit series demonstrates the inherent value of Muller's morphs (hypo-, hyper-, neo- and antimorphs) for high-resolution genetic analyses of molecular pathways. Furthermore, allelic series often provide better models of human disease, where phenotypes are rarely the result of null or amorphic alleles. Forward genetics (phenotype driven approaches) remain the primary source for non-null alleles. Unfortunatley, the gap between phenotype and genotype limits the widespread use of spontaneous and induced mouse mutants. While the current positional cloning process is straightforward, it requires some facility in mouse genetics, ample vivarium space and it is labor intensive because it relies heavily on PCR amplification of large numbers of individual templates for Sanger sequencing. Array enrichment and next generation sequencing technology can be used to rapidly sequence subsets of the genome. Recently, array capture has been used to sequence the coding portion of the human genome. This technology has the potential to significantly reduce the time and resources required for mutation identification by abrogating the need for high-resolution genetic mapping, long range PCR, and sequencing of individual PCR amplimers. As proof of principle that array enrichment and next generation sequencing technology can be used to *rapidly* identify mutations, we have used these technologies sequence ~200 kb of genomic DNA from each of 5 *Kit* heterozygotes (one known allele and 4 previously unknown alleles, 1 Mb total) and we have successfully identified and validated a non-synonymous coding mutation for each. These data demonstrate that these new technologies can be used to significantly close the gap between phenotype and genotype in the mouse.

OTHER GENOMES AND NEW APPROACHES
ORAL PRESENTATION TUESDAY NOVEMBER, 4 4.15PM – 4.30PM

O-28 THE COMPLETE SEQUENCE OF MOUSE CHROMOSOME 17 FROM TWO INBRED MOUSE STRAINS; *A/J* AND *CAST/EI*

Jim Stalker[1], Ian Sudberry[1], Bee Ling Ng[1], Lydia Teboul[2], Dee Lynch[2], Steve Brown[2], David Adams[1]
[1]Wellcome Trust Sanger Institute, Cambridgeshire, United Kingdom, [2]MRC-Harwell, Oxfordshire, United Kingdom

The complete sequence of all mouse strain genomes will significantly improve the power of genetic screens in mice, facilitate comparative genomics and the rapid identification of disease causing variants, modifiers and QTLs. Here we use Illumina sequencing technology to rapidly re-sequence chromosome 17 from two different mouse strains, the laboratory strain *A/J,* and the wild-derived strain *CAST/Ei* of the *Mus musculus castaneus* sub-species, to a high sequence depth. By aligning the sequences generated to the *C57BL/6J* reference genome assembly we identified the vast majority of SNPs found in the dbSNP database, and >99.9% of an experimentally verified mouse SNPs collection. In addition we found a large number of novel SNPs, a number of which we predict will cause the loss or gain of stop codons or the substitution of amino acids in coding sequences. A sample of these SNPs were validated using an independent genotyping assay. By sequencing these chromosomes we have also identified a large number of copy number variants on mCh17 in these strains. This project demonstrates the viability of using Illumina sequencing to rapidly and accurately sequence whole mouse chromosomes using approaches that should be scalable to whole mouse genomes, thus making it possible to generate a comprehensive inventory of the differences between mouse strains.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 9.00AM – 9.15AM

O-29 ABERRANT AGOUTI-RELATED PROTEIN SYSTEM IN THE HYPOTHALAMUS OF THE *ANX/ANX* MOUSE IS ASSOCIATED WITH ACTIVATION OF MICROGLIA

Ida Nilsson[1], Charlotte Lindfors[1], Serguei Fetissov[2], Tomas Hökfelt[3], Jeanette Johansen[1]
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Mice homozygous for the anorexia (*anx*) mutation, are used for studying regulation of food intake and are characterized by decreased food intake, starvation and death by three to five weeks of age. A key food intake stimulating neuropeptide expressed in the hypothalamic arcuate nucleus is Agouti-related protein (AgRP), often used as marker for neurons conveying hormonal signals of hunger to the brain. By week three immunoreactivity for AgRP is increased in cell bodies but decreased in the nerve terminals of *anx/anx* mice. We studied, when during early postnatal development the aberrant phenotype of the AgRP system becomes apparent in *anx/anx* mice, and possible underlying mechanisms. AgRP and ionized calcium binding adapter molecule (Iba1), a marker of activated microglia, were studied by immunohistochemistry at postnatal days P1, P5, P10, P12, P15 and P21 in *anx*/*anx* and wild type mice. We found that the AgRP system in *anx/anx* mice develops similarly to wild type up till P12, when AgRP fibers in *anx/anx* mice cease to increase in density in the main projection areas. At P21, AgRP fiber density in *anx/anx* mice was significantly reduced vs. P15, in certain regions. At P21 many strongly AgRP-positive cellbodies were observed in the *anx/anx* arcuate nucleus, versus only few and weakly fluorescent ones in the wild type. The decrease in AgRP fiber density in *anx/anx* mice overlapped with increased Iba1 immunoreactivity. Thus, the aberrant appearance of the AgRP system in the *anx/anx* mouse in the early postnatal development is related to a microglia-associated process e.g inflammation/degeneration.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 9.15AM – 9.30AM

O-30 GENETIC AND PHENOTYPIC STUDIES OF THE DARK-LIKE MUTANT MOUSE

Christina Cota, Roy Liu, Teresa Gunn
Cornell University, Ithaca, NY, United States

Mouse pigmentation has a rich history of providing insight into fundamental signaling pathways that underlie many physiological processes. *Dark-like* (*dal*) mutant mice display a pleiotrophic phenotype that includes reproductive degeneration and a darkened coat similar to that of *Attractin* (*Atrn*) mutants. We further characterized the reproductive defects in *dal* mutant males and examined the genetic and phenotypic interactions of *dal* with other genes involved in pigment-type switching. Histological analysis revealed significant vacuolation within the seminiferous tubules of 3 month-old *dal* mutant males. These vacuoles were similar in appearance to those that develop in the brains of *Atrn* mutant mice. Genetic crosses were used to position *dal* in the pigment-type switching pathway, upstream of the *Melanocortin 1 receptor* (*Mc1r)* and downstream of *agouti* transcription. Interestingly, unlike *Atrn*, the *dal* mutation suppressed the effects of ectopic agouti expression pigmentation but not body weight. $Atrn^{mg-3J}$, an *Atrn* null allele, showed additive effects with *dal* on pigmentation, testicular vacuolation and spongiform neurodegeneration, but transgenic over-expression of *Atrnl1* (which compensates for loss of ATRN) did not rescue *dal* mutant phenotypes. Our results suggest that *dal* and ATRN function in the same cellular pathway and that identification of the *dal* gene will provide insight into molecular mechanisms of spongiform degeneration in multiple cell types.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 9.30AM – 9.45AM

O-31 HIGH PHENOTYPIC DIVERSITY FOR METABOLISM AND VOLUNTARY EXERCISE IN EMERGING LINES OF THE COLLABORATIVE CROSS

Wendy Foulds Mathes[1], Elissa Chesler[2], Fernando Pardo-Manuel de Villena[1], David Threadgill[1], Daniel Pomp[1]
[1]University of North Carolina, Chapel Hill, NC, United States, [2]Oak Ridge National Laboratories, Oak Ridge, TN, United States

The Collaborative Cross (CC) is an innovative research tool designed to model genetic diversity of human populations. Originating from crossing of eight genetically diverse founder strains, the CC is a collection of recombinant inbred mouse lines that are infinitely reproducible. Once breeding is complete, each line will comprise approximately 135 recombination events and overall will have segregating polymorphisms every 75 base pairs. Relative to existing resources, this breadth of genetic diversity will greatly enable the study of genetic proclivity, gene by environment interactions, and gene-gene interactions for complex traits. An initial phenotyping experiment has revealed profound differences among the emerging CC mouse lines (i.e., the “preCC”). Adult male mice (10-13 wk) were evaluated for body weight, body composition, food intake, metabolic rate and wheel running. The results show that the individual lines of preCC mice span the same magnitude of phenotypic diversity as they do genetic diversity. For example, initial body weights and body fat percentages varied from 16.2-39.0 g and 8-40%, respectively. Diurnal respiratory exchange ratios (RER) spanned from 0.82-1.03 while nocturnal RERs ranged from 0.91-1.16. Wheel running increased gradually over time, culminating in distances ranging from 1.6 to 17.5 kilometers during the final 24 g of measurement. The genotypic and phenotypic diversity exemplified within the preCC demonstrate that the CC will be an invaluable tool for identification of genes underlying complex traits and for understanding the genetic architecture of disease etiology.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 9.45AM – 10.00AM

O-32 EFFECTS OF JARID1C SIRNA TREATMENT ON OBJECT RECOGNITION IN MICE

Marc Siegel, Elizabeth Byrnes, Jun Xu
Tufts University Cummings School of Veterinary Medicine, North Grafton, MA, United States

Jarid1c is an X-linked gene coding for a demethylase enzyme specific for histone H3 lysine 4. In humans, mutations of *JARID1C* cause X-linked mental retardation, as well as aggression and epilepsy. To identify genes regulated by *Jarid1c* and brain regions where *Jarid1c*'s regulatory effects take place, we performed RNA interference-mediated gene silencing in mice. *Jarid1c*-specific siRNAs or control siRNAs were injected into the dorsal hippocampus, a brain region known to be involved in learning and memory. Two days later, the treated mice were tested using an object recognition task. Mice treated with *Jarid1c* siRNA tended to demonstrate impaired memory formation. Gene expression was also analyzed using Mouse GeneChip. The majority of genes were not affected by the *Jarid1c* siRNA treatment. Among genes found to be down-regulated, many encode genes known to be implicated in neural plasticity. These preliminary findings suggest that *Jarid1c* likely regulates specific genes in the hippocampus and in turn affects certain cognitive processes.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 10.00AM – 10.15AM

O-33 BEHAVIORAL EFFECTS OF A DELETION IN *KCNN2*, THE GENE ENCODING THE SK2 SUBUNIT OF SMALL-CONDUCTANCE CA^{2+}-ACTIVATED K^+ CHANNELS

Marek Szatanik[1], Nicolas Vibert[2], Isabelle Vassias[2], Jean-Louis Guénet[1], Daniel Eugène[2], Catherine de Waele[2], Jean Jaubert[1]
[1]Institut Pasteur, PARIS, France, [2]CNRS-Centre Universitaire des Saints-Pères, PARIS, France

Small-conductance Ca^{2+}-activated potassium (SK) channels are heteromeric complexes of SK alpha-subunits and calmodulin that modulate membrane excitability, are responsible for part of the after-hyperpolarization (AHP) following action potentials, and thus control the firing patterns and excitability of most central neurons. An engineered knock-out allele for the SK2 subunit has previously been reported. The hippocampal neurons of these mice lacked the medium latency component of the AHP, but the animals were not described as presenting any overt behavioral phenotype. In this report, we describe a deletion in the 5' region of the *Kcnn2* gene encoding the SK2 subunit in the mouse neurological *frissonnant* (*fri*) mutant. The *frissonnant* mutant phenotype is characterized by constant rapid tremor and locomotor instability. It has been suggested, based merely on its phenotype, as a potential model for human Parkinson Disease. We used a positional cloning strategy to identify the mutation underlying the *frissonnant* phenotype. We narrowed the genetic disease interval and identified a 3441bp deletion in the *Kcnn2* gene, one of the three candidate genes present in the interval. Expression studies showed complete absence of normal *Kcnn2* transcripts while some tissue–specific abnormal truncated variants were detected. Intracellular electrophysiological recordings of central vestibular neurons revealed permanent alterations of the AHP and firing behavior that might cause the tremor and associated locomotor deficits. Thus, the *fri* mutation suggests a new, potentially important physiological role, which had not been described, for the SK2 subunit of small-conductance Ca^{2+}-activated potassium channels.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 10.15AM – 10.30AM

O-34 MICE DEFICIENT IN *ALIVIN1/AMIGO2* SHOW ENHANCED LOCOMOTOR ACTIVITY AND REDUCED FEAR AND ANXIETY

Tomio Ono[1], Noriko Akamatsu[1], Hiroshi Shitara[1], Rie Ishii[1], Choji Taya[1], Ikuko Yamada[2], Yoko Shibukawa[2], Tomoko Kushida[2], Tamio Furuse[2], Kazuhiko Watabe[3], Shigeharu Wakana[2], Hiromichi Yonekawa[1]
[1]Lab. of Mouse Models for Human Heritable Diseases, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [2]Technology and Development Team for Mouse Phenotype Analysis, RIKEN BRC, Tsukuba, Japan, [3]Dept. of Molecular Neuropathology, Tokyo Metropolitan Institute for Neuroscience, Tokyo, Japan

Alivin1/amigo2 is a neuronal activity-dependent gene that is a potential therapeutic target for neurodegenerative diseases. This gene was originally discovered by us (1) and has been shown to be involved in suppression of apoptosis, cell adhesion, migration, and carcinogenesis. *Alivin1* encodes a transmembrane protein that has 7 leucine-rich repeats (LRR) and one immunoglobulin (IG) domain. The encoded protein is assumed to interact with its binding partner(s) through these LRR and IG domains and involve in the signal transduction(s). Real time RT-PCR analysis revealed that the expression of *alivin1* mRNA is higher in brain, lung, and spleen among the mouse tissues examined.

In order to study biological functions of *alivin1* in mice, we generated the knockout mouse line. Open-field tests were carried out twice with a week interval using the same subjects of *alivin1* (-/-) and wild type mice (WT). The primary open-field tests showed that the per cent time spent in the central area of the field of *alivin 1* (-/-) mice was larger than that of WT, suggesting that *alivin 1* (-/-) mice have reduced fear and anxiety compared to WT. The comparison between the results of the primary and the secondary tests showed that the locomotor activity of *alivin1* (-/-) mice under the familiar environment was larger than that of WT. *Alivin1* (-/-) mice kept in their home cages also showed higher locomotor activity than WT. These results suggest that deficiency in *alivin1* is associated with enhanced locomotor activity and reduced fear and anxiety.

(1) Ono *et al.* J. Neurosci. 23, 5887-5896, 2003.

NEUROSCIENCE/BEHAVIOR
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 10.15AM – 10.30AM

O-35 *MELODY* AN ENU MUTATION IN *CASPASE-3*, CAUSES SENSORINEURAL HEARING LOSS IN MICE

Andrew Parker, Susan Joyce, Emma Coghill, Steve D M Brown, Rachel E Hardisty-Hughes
MRC Mammalian Genetics Unit, Harwell, United Kingdom

G3 progeny from the Harwell *N*-ethyl-*N*-nitrosourea (ENU) recessive screen were assessed for auditory defects. During this screen a pedigree was identified with multiple progeny lacking in response to a ‘clickbox’ (20kHz tone, 90dB SPL). Subsequent Auditory Brainstem Response (ABR) analysis showed that these mice were profoundly deaf.

We subsequently mapped this mutation to a ~23 Mb region on Chromosome 8. We have identified a point mutation in the *melody* line that results in a C163S substitution in the catalytic site of *Caspase-3*, a cysteine protease involved in apoptosis. Histological analysis has shown degeneration of spiral ganglion cells in homozygote mice. Scanning Electron Microscopy (SEM) has revealed disorganised sensory hair cells and hair cell loss. Work is currently being undertaken to learn more about possible functional effects of this mutation.

MODELING DISEASE
ORAL PRESENTATION **WEDNESDAY NOVEMBER, 5** **1.45PM – 2.00PM**

O-36 THE CONSTRUCTION AND ANALYSIS OF A MOUSE GENE-TRAP MUTANT RESOURCE CREATED IN THE C57BL/6N GENETIC BACKGROUND

Gwenn M. Hansen[2], Diane Markesich[2], Michael McLeod[1], Andrei Golovko[1], Richard H. Finnell[1]
[1]Texas Institute for Genomic Medicine, Houston, TX, United States, [2]Lexicon Pharmaceutical Incorporated, The Woodlands, TX, United States

Here we report the first large-scale use of a C57BL/6 ES cell line in the production of a library of mutagenized ES cell clones for generating knock-out mice. We have used high-throughput gene-trapping with retroviral vectors in mouse C57BL/6N ES cells to generate a library of more than 480,000 mutated ES cell clones. We generated a tractable and unique sequence tag from 73% of the clones that was subjected to an automated inverse genomic PCR-based direct-sequencing protocol. As of today, TIGM resource contains over 270,000 sequence-tagged ES cell clones suitable for producing gene knockouts. Each mutant clone is identified by a genomic sequence tag representing the exact insertion site, allowing accurate prediction of mutagenicity and enabling direct genotyping of mutant alleles. Mutations have been identified in over 10,000 genes and show a bias toward the first gene intron.
Mutant clones demonstrated moderate performance in blastocysts microinjections. The average injection success rate of individual clones of the library was about 64%. On a clone by clone basis, the average germline transmission rate achieved from each of the C57BL/6N clone projects was 43% for coat color transmission.
The trapped ES cell lines, which can be requested from the Texas Institute for Genomic Medicine (TIGM; www.tigm.org), are readily available to the scientific community.

MODELING DISEASE
ORAL PRESENTATION **WEDNESDAY NOVEMBER, 5** **2.00PM – 2.15PM**

O-37 MANUAL ANNOTATION OF THE MOUSE GENOME AND EUCOMM

Mark Thomas, Clara Amid, Denise Carvalho-Silva, Vivek Iyer, Manousos Koutsourakis, Jane Loveland, Alejandro Mujica, Jeena Rajan, Catherine Snow, Marie-Marthe Suner, Jennifer Harrow, Bill Skarnes, Tim Hubbard, Alan Bradley
Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom

The Wellcome Trust Sanger Institute (WTSI) is a member of the European Conditional Mouse Mutagenesis (EUCOMM) project, which aims to establish a resource containing up to 20,000 conditional mouse mutations in embryonic stem (ES) cells. As part of this resource, EUCOMM intends to create up to 8,000 targeted conditional mutations for genes, which can not be readily trapped by random gene trapping methods. This targeting approach involves the identification of a non-symmetrical critical exon, which when deleted produces a knockout (KO) transcript that is susceptible to nonsense-mediated decay (NMD). The success of this approach is dependent on detailed and accurate genome annotation. This annotation is provided by the Human and Vertebrate Analysis and Annotation (HAVANA) group at the WTSI. Based on a combination of EST, cDNA and protein evidence, we produce high quality manual annotation of gene structures, including alternative splice variants, poly-adenylation features, pseudogenes and gene family clusters. Using this annotation we are able to identify suitable exons for targeting, whilst minimising potential disruption to adjacent genomic features.
Annotation of both the gene and KO transcripts can be viewed through the Vertebrate Genome Annotation (VEGA) browser (http://vega.sanger.ac.uk). Targeting vector designs are currently available in the Ensembl genome browser (http://www.ensembl.org) as DAS resources (http://das.sanger.ac.uk/das/KO_designs; http://das.sanger.ac.uk/das/KO_vectors). The HAVANA group is also involved in collaborations with RefSeq and MGI and contributes towards the mouse Consensus Coding DNA Sequence (CCDS) project.

MODELING DISEASE
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 2.15PM – 2.30PM

O-38 THE KNOCKOUT MOUSE PROJECT DATA COORDINATION CENTER (KOMP-DCC)

Carol Bult, Janan Eppig, James Kadin, Jeremy Mason, Hamsa Tadepally, Martin Ringwald
The Jackson Laboratory, Bar Harbor, ME, United States

The NIH funded Knockout Mouse Project (KOMP), together with the other members of the International Knockout Mouse Consortium, aims to generate a public resource of mouse embryonic stem (ES) cells containing a null mutation in every gene in the mouse genome. Using complementary targeting strategies, two KOMP Production Centers, Regeneron Pharmaceuticals, Inc. and CSD, a collaborative team at the Children's Hospital Oakland Research Institute (CHORI), the Wellcome Trust Sanger Institute, and the University of California at Davis School of Veterinary Medicine (UC Davis), design and create targeting vectors, mutant ES cell lines and, to some extent, mutant mice. These KOMP products are being maintained by and can be obtained from the KOMP Repository (http://www.komp.org).

The KOMP Data Coordination Center is the central database resource for coordinating mouse gene targeting within KOMP. Data and tools provided by the DCC are used by KOMP to prioritize new mouse genes for knockout experiments and assign them to KOMP production centers. The DCC tracks progress of the knockout production pipelines, collects, standardizes, and integrates KOMP data and makes the data available to all KOMP participants. The DCC also serves as the central public interface for the KOMP and provides web-based query and display tools for KOMP data. In addition, the website provides a tool for the scientific community to nominate genes of interest to be knocked-out by the KOMP. The current status of the KOMP-DCC work will be presented. The KOMP-DCC web site is accessible at http://www.knockoutmouse.org. The KOMP-DCC is supported by NIH grant HG004074.

MUTAGENESIS
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 3.30PM – 3.45PM

O-39 ENU-INDUCED MUTATIONS CAUSING ARREST OF SPERMATOCYTES BEFORE THE FIRST MEIOTIC METAPHASE

Fengyun Sun[1], Kristina Palmer[1], Kaitlin Laws[1], John Schimenti[2], Mary Ann Handel[1]
[1]The Jackson Laboratory, Bar Harbor, Maine, United States, [2]Cornell University, Ithaca, New York, United States

In the ReproGenomics program at The Jackson Laboratory, ENU mutagenesis and phenotype screens for infertility have identified two new mouse mutations, *repro4* and *repro8*, affecting transition from meiotic prophase I to metaphase I in spermatocytes. Homozygous males are characterized by arrest of spermatogenesis in prophase; for *repro8*, the arrest is in the late pachytene stage and for *repro4*, the arrest is at diplonema. Analysis of whole-mount nuclei and surface-spread chromatin revealed normal chromosome synapsis and normal localization of meiotically relevant proteins to the synaptonemal complex in both *repro4* and *repro8* mutant spermatocytes. Presence of the mid-pachytene marker, histone H1t in mutant spermatocytes also confirmed developmental progress to mid or late pachynema. Although mutant spermatocytes did not reach metaphase, many proteins characteristic of the late prophase-metaphase transition were not obviously different in mutant spermatocytes. When treated in vitro with okadaic acid to induce transition from meiotic prophase to metaphase, mutant, as well as wild type, spermatocytes phosphorylated histone H3, an event that ordinarily marks transition to metaphase. Nonetheless, mutant spermatocytes, unlike wild type spermatocytes, did not condense normally compacted metaphase chromosomes when treated with okadaic acid. These two mutations thus identify two genes encoding proteins essential for meiotic progress and entry into division phase. Genetic fine mapping localized the *repro4* mutation to Chr 1 and the *repro8* mutation to Chr 4; a strong candidate gene has been identified for each mutation. (Supported NIH grants HD42137 and HD33816)

MUTAGENESIS
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 3.45PM – 4.00PM

O-40 THE SPLICE SITE MUTATION *GOYA* IN *MAP3K1* CAUSES EYE DEFECTS AND DEAFNESS IN THE MOUSE

Sally Cross[1], Rachel Hardisty-Hughes[2], Russell Joynson[2], Katrine West[1], Lisa Mckie[1], Emma Coghill[2], Andrew Parker[2], Steve Brown[2], Ian Jackson[1]
[1]MRC Human Genetics Unit, Edinburgh, Lothian, United Kingdom, [2]MRC Mammalian Genetics Unit, Harwell, Oxfordshire, United Kingdom

We are conducting a three generation screen for recessive ENU-induced mutations that cause eye and vision defects in the mouse. Mice homozygous for the mutation *goya* are born with their eyes open. Mutant adult eyes are highly variable, ranging in pathology from apparently normal to bulging to microphthalmic. However, irrespective of eye phenotype *goya* mice fail to respond in a visual function assay. Additionally, mutants lack a Preyer response to auditory stimulus and their auditory brainstem response is impaired. At a gross level mutant ears appear normal. Electron microscopic analysis of the inner ear reveals that the number of outer hair cells is greatly reduced. Only short stretches of inner hair cells are present with abnormal bundle morphology. We mapped *goya* to an 8.6 Mb region on chromosome 13 using five homozygotes and a panel of 377 SNPs. *Map3k1* located in this interval is a strong candidate gene because mice deficient for *Map3k1* have open eyes at birth. Sequencing *Map3k1* in *goya* mice found a single nucleotide change in the intron 13 splice donor site (IVS13+2T>C). RT-PCR analysis showed that this generates two abnormal transcripts. One skips exon 13 creating a transcript with a frameshift that would yield a C-terminally truncated protein if translated. The second utilises a cryptic splice site in exon 13 and would be predicted to produce an aberrant protein with an internal deletion of 27 amino acids. Deafness has not been noted in previously characterised *Map3k1* alleles. Investigations into the aetiology of these mutant phenotypes continue.

MUTAGENESIS
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 4.00PM – 4.15PM

O-41 DOSE-DEPENDENT EFFECT OF ZINC-FINGER TRANSCRIPTION FACTOR BCL11B ON DIFFERENTIATION OF CYTOTOXIC T CELLS

Ryo Kominami[1], Satoshi Hirose[1], Ryota Ishizawa[1], Yoshinori Katsuragi[1], Yoshiyuki Sakuraba[2], Yoichi Gondo[2]
[1]Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan, [2]RIKEN BioResource center, Tsukuba, Japan

CD4/CD8 double positive (DP) thymocytes undergo positive selection, differentiating into either CD8SP cytotoxic or CD4SP helper T cells. Upon TCR signaling, DP cells initiate a differentiation program that induces complex changes in CD4 and CD8 expression. A current paradigm of transitional intermediates to CD8SP is as follows. DP cells give rise to $CD4^{+}CD8^{low}$ cells, not yet committed to either lineage, and path through two more stages, $CD8^{low}CD4^{low}$ and $CD8^{+}CD4^{low}$, before becoming CD8SP cells. *Bcl11b* is a haploinsufficient tumor suppressor and plays an essential role for abT cell development. *Bcl11b* knockout mice exhibit a block at developmentally early stage and do not generate DP thymocytes. On the other hand, *Bcl11b* heterozygous mice show retardation of thymocyte development in embryos. This suggests its dose-dependent effect on differentiation of thymocytes and possibly CD4SP and CD8SP cells. To analyze this dose-dependency, we generated mice carrying the hypomorphic allele of *Bcl11b* by ENU mutagenesis. Among six such mutants analyzed, one (1891mut) carrying one-base substitution leading to a missense serine to glycine substitution at codon 826 in the C-terminal zinc finger motif. Here, we show a developmental impairment at the $CD8^{low}CD4^{low}$ transitional stage of positive selection caused by the 1891mut allele. As a result, development of CD8SP cells, but not of CD4SP, was reduced in 1891mut/+ mice. This reduction was more sever in KO/+ mice. Our data suggest that differentiation of CD8 lineage T cells is controlled by Bcl11b and its processing level is affected by or dependent on Bcl11b activity.

MUTAGENESIS
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 4.15PM – 4.30PM

O-42 GENETIC AND PHENOTYPIC ANALYSES OF AN ENU-INDUCED MOUSE MUTANT THAT SHOWS AD/HD-LIKE BEHAVIOR

Tamio Furuse[1], Yumiko Wada[2], Kotaro Hattori[3], Ikuko Yamada[1], Tomoko Kushida[1], Yoko Shibukawa[1], Hiroshi Masuya[1], Hideki Kaneda[1], Ikuo Miura[1], Kimio Kobayashi[1], Toshihiko Shiroishi[4], Shigeki Yuasa[3], Shigeharu Wakana[1]
[1]RIKEN BRC, Tsukuba, Ibaraki, Japan, [2]Health Science University, Kawaguchiko-cho, Yamanashi, Japan, [3]National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan, [4]National Institute of Genetics, Mishima, Shizuoka, Japan

In RIKEN large-scale ENU mutagenesis project, we are carrying out behavioral screening to develop novel models for psychiatric disorders. In dominant screening, a mutant mouse M-174 that showed hyper-locomotor activity in open-field test was isolated from G1 population. In linkage analysis, causative locus was mapped to proximal region of chromosome2. In detailed linkage analysis, the responsible mutation for hyper activity was mapped between D2Mit81 and D2Mit521. As a result of genome sequence analyses of candidate genes, we identified a missense mutation with amino acid substitution in the C0 domain of Grin1 gene that encodes NMDA receptor subunit 1 (NMDAR1). We conducted comprehensive phenotype analyses of the M-174 mutant mouse, which include general histology of brain tissue, home-cage activity test, social interaction, motor coordination, object exploration test, and pharmacological analyses with effects of methylphenidate (MPH) treatment in the open-field activity. Results of these tests indicate that M-174 mutant line is a candidate for an animal model of attention deficit/hyperactivity disorder (AD/HD). In further analyses, c-Fos expression pattern and ERK phosphorylation level in the brain tissue were examined using immunohistochemical staining and western blot. All the results indicated that M-174 would be a useful unique animal model for elucidating pathology of AD/HD.

MUTAGENESIS
ORAL PRESENTATION WEDNESDAY NOVEMBER, 5 4.30PM – 4.45PM

O-43 A SENSITIZED MOUSE MUTAGENESIS SCREEN FOR MODIFIERS OF SOX10 NEUROCRISTOPATHIES

Dawn Watkins-Chow[1], Denise Larson[1], Debra Silver[1], Kristina Buac[1], Ivana Matera[2], Stacie Loftus[1], William Pavan[1]
[1]NIH, NHGRI, Bethesda, MD, United States, [2]Instituto G. Gaslini, Genova, Italy

The neural crest is a pluripotent cell population that arises during mammalian development and gives rise to a variety of cell types including cartilage, bone, melanocytes of the skin, and neurons and glia of the peripheral nervous system. Disrupting the normal development of these lineages can cause debilitating diseases, collectively referred to as neurocristopathies, that present with a variety of phenotypes including deafness, blindness, cleft lip, congenital megacolon, and albinism. As genetic background is known to affect the severity of neurocristophathies in both humans and mice, we have established an enhancer screen to identify mutations that increase the phenotypic severity of *Sox10* haploinsufficient mice ($Sox10^{LacZ/+}$), a well-characterized mouse model of human neurocristopathies. In analysis of 400 pedigrees, we have identified four dominant modifiers of *Sox10* neurocristopathies (*Mos1-4)* and four recessive phenotypes affecting embryonic $Sox10^{LacZ}$ expression (*msp1-4*). The causative mutations affect genes involved in a variety of functions including hedgehog, neuregulin, and semaphorin signaling as well as ribosomal and RNA binding proteins. The phenotypes we have identified do not overlap previously known major mouse spotting loci, thus demonstrating the feasibility of this screen to provide a more detailed understanding of the critical genes regulating mammalian neural crest development and to provide additional disease models for human neurocristopathies.