



Saturday November 5
Oral Presenters Abstracts

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Abstracts O-1 – O-41

Genome Sequencing and Comparative Analysis**Oral Presentation****Saturday November 5****2.30pm – 2.45pm****O-1****PROMOTING MAMMALIAN TRANSCRIPTION**

P Carninci², A Sandelin¹, B Lenhard³, S Katayama¹, K Shimokawa¹, J Ponjavic¹, C A M Semple⁴, M S Taylor¹, V B Bajic⁷, D A Hume⁶, Y Hayashizaki¹

¹ Genome Exploration Research Group, RIKEN Genomic Sciences Center (GSC), Yokohama, Kanagawa, Japan, ² Genome Science Laboratory, Discovery and Research Institute, RIKEN Wako Institute, 2-1 Hirosawa, Wako, Saitama, Japan, ³ Center for Genomics and Bioinformatics, Karolinska Institutet, Stockholm, Sweden, ⁴ MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom, ⁵ University of Oxford, Oxford, United Kingdom, ⁶ ARC Special Research Centre for Functional and Applied Genomics, Institute for Molecular Bioscience, The University of Queensland, Brisbane Qld, Australia, ⁷ Knowledge Extraction Laboratory, Institute for Infocomm Research, Singapore,

To better understand transcription regulation, we have systematically isolated and sequenced CAGE (cap-analysis gene expression) tags corresponding to the 5'-ends of mRNAs in order to map transcription start sites (TSSs) and core promoters in the mouse and human genomes, and to analyze their structure, evolution and diversity. We have produced ~8.9 million CAGE tags from mouse and ~5.5 million CAGE tags from human, to identify 236,498 mouse and 190,513 human TSSs.

We have then produced for the first time a comprehensive hierarchical clustering based on TSS usage. Surprisingly, different classes of promoters determine transcription of different type of RNA transcripts. Our analysis shows that mammalian promoters can be separated into two classes, conserved TATA box enriched promoters, which initiate at a well-defined site, and more plastic, broad and evolvable CpG-rich promoters. Different tissues and families of genes make differential use of these types of promoters.

Our tagging methods allow quantitative analysis of promoter usage in different tissues, and reveal that differentially regulated alternative TSSs are a common feature in protein coding genes. We also identify novel motifs responsible for specific transcription of certain non-coding RNAs. The data can be used to identify tissue-specific promoters, and to analyze the cis-acting elements associated with them. As an example of the utility of the data set, we identify the transcription factors associated with promoters during macrophage differentiation and activation by lipopolysaccharide.

Our data provide the most comprehensive promoter map in mammalian, which paves the way for transcriptional network analysis and systematic system biology.

Genome Sequencing and Comparative Analysis**Oral Presentation****Saturday November 5****2.45pm – 3.00pm****O-2****SINGLE MOLECULE ANALYSIS TO RESOLVE UNASSEMBLED REGIONS OF THE B6 GENOME**

J Amos-Landgraf¹, S Goldstein², S Zhou², J Herschleb², B Teague², L N Kwong², A Shedlovsky¹, W F Dove¹, D C Schwartz²

¹ McArdle Cancer Research Laboratories, University of Wisconsin, Madison, WI, United States, ² Laboratories for Molecular and Computational Genomics, University of Wisconsin, Madison, WI, United States

With the latest assembly of the mouse genome sequence, most of the unique regions of the genome have been resolved; however, repetitive elements have remained refractory to traditional assembly methods. Such repetitive sequences have been implicated in evolution, disease breakpoints, and as recombinogenic substrates. The availability of an optical map covering ~95% of the mouse genome now enables analysis of previously inaccessible regions within the genome. The analysis platform utilizes hundreds of thousands of half-megabase-sized genomic DNA molecules that are individually bar-coded by restriction digestion and then assembled into contigs spanning the entire genome. Alignments of these map contigs with Build 34 sequence has led to the identification of regions of misassembly and putative forms of variation. The maps bridge ~14000 sequence gaps <50 kb and ~250 gaps >50kb, providing an initial visualization of many previously uncharacterized regions. Importantly, optical maps extend an average of ~250 kb on 14 chromosomes into unassembled pericentromeric and telomeric regions. The applicability of such direct visualization can be further illustrated by our analysis of an intestinal cancer modifier, which maps to the pericentromeric region of chromosome 18. Unexpectedly, intrastrain variation was found between the current JAX B6 stock and Build 34 genome sequence located within the pericentromeric region of chromosome 18 and other regions of the genome. Optical mapping provides a necessary approach not only to examine repetitive regions of the genome, but also to assess genome wide germline isogenicity and somatic rearrangements in a small number of tumor cells.

Genome Sequencing and Comparative Analysis**Oral Presentation****Saturday November 5****3.00pm – 3.15pm****O-3****HIGH-RESOLUTION MAPPING OF RECOMBINATION ON MOUSE CHROMOSOME 1**

P M Petkov, K Sawyer, L Guccione, K Paigen
The Jackson Laboratory, Bar Harbor, ME, United States

Although it has been known for some years that mammalian meiotic recombination occurs at special sites termed hotspots, rather than occurring randomly along the chromosomes, we know little about the genomic organization of this important biological process. To address this lack, we have constructed the first large-scale, detailed recombination map of a mammalian chromosome using 6000 meioses from crosses of C57BL/6 x Cast/EiJ. 100 Mb of Chr 1 were mapped at 5 Mb resolution, within this 25 Mb at approx 100 Kb resolution and 5 Kb at even greater resolution. We can draw several conclusions from the resulting maps.

Hot spots vary greatly in activity, and all activity classes are nearly equally represented, with only a slight bias against the most active hotspots. As a result, most recombination occurs at very few sites.

Hot spots are not randomly distributed along the chromosome. There are large regions, one Mb or more, with very little or no recombination and “torrid zones” where high activity hotspots are clustered. The result of this is a very “granular” genetic map.

The 1.3X increased recombination on autosomes in females v. males is largely not due to the presence of additional female specific hotspots; the two sexes share the same hotspots which differ in activity in male and female meiosis, although sex-specific recombination sites cannot be ruled out. Surprisingly, the female/male ratio is regionally determined, rather than being an individual property of each hotspot. This last fact requires that we introduce new parameters into our models of mammalian recombination.

Genome Sequencing and Comparative Analysis**Oral Presentation****Saturday November 5****3.15pm – 3.30pm****O-4****IMGT OVERVIEW: 8. MOUSE IMMUNOGLOBULIN AND T CELL RECEPTOR GENES AND IMGT-ONTOLOGY FOR IGSF AND MHCSF**V Giudicelli, M-P Lefranc

IMGT, the international ImMunoGeneTics information system®, LIGM, UM2, IGH, Montpellier, France

Except for 24 IGHV genes not yet identified, all the immunoglobulin (IG) and T cell receptor (TR) genes in *Mus musculus* laboratory mice are known: there are 610-614 genes (372-376 IG and 238 TR) in the seven main loci, and 10 IGKV orphans. The number of functional genes is 412-432, comprising 242-250 IG genes and 170-182 TR genes (detailed in the accompanying abstracts). These data result from an exhaustive analysis in IMGT, the international ImMunoGeneTics information system®, <http://imgt.cines.fr> [1], based on the IMGT-ONTOLOGY concepts [2]. The assignment of a sequence to a 'locus', 'group', 'subgroup', 'gene' and 'allele' in IMGT/GENE-DB relies on the 'CLASSIFICATION' concept. The sequence and domain motifs in IMGT/LIGM-DB (22,219 mouse IG and TR sequences) and in IMGT/3Dstructure-DB (544 mouse IG, TR and MHC three-dimensional structures) are described with standardized labels according to the 'DESCRIPTION' concept. Codon and amino acid numbering is based on the 'NUMEROTATION' concept. Interestingly these concepts, and particularly the IMGT unique numbering, initially set up for the domains of the IG, TR and MHC have been extended to the V-LIKE-DOMAIN [3] and C-LIKE-DOMAIN [4] of the IgSF other than IG and TR, and to the G-LIKE-DOMAIN [5] of the MhcSF other than MHC, as illustrated by the IMGT Collier de Perles [6].

[1] Nucl. Acids Res. 33, D593-D597 (2005). [2] Bioinformatics 15, 1047-1054 (1999). [3] Dev. Comp. Immunol. 27, 55-77 (2003). [4] Dev. Comp. Immunol. 29, 185-203 (2005). [5] Dev. Comp. Immunol. 29, 917-938 (2005). [6] In Silico Biology 5, 45-60 (2005).

Genome Sequencing and Comparative Analysis

Oral Presentation

Saturday November 5

4.00pm – 4.15pm

O-5

THE CHARCOT-MARIE-TOOTH DISEASE AND SMITH-MAGENIS SYNDROME REGION: COMPARING MOUSE CHROMOSOME 11 AND HUMAN CHROMOSOME 17

L G Wilming, D Adams, R C Gibson, J G R Gilbert, E A Hart, G K Laird, J E Loveland, J Mudge, S Searle, C A Steward, D Swarbreck, J L Harrow, T Hubbard
Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, United Kingdom

Mouse chromosome 11 is the first mouse chromosome to be finished with clone based sequence. This allows for a more detailed and accurate annotation, especially of repetitive, re-arranged and polymorphic regions, than whole genome shotgun sequence. Most of the chromosome is syntenic to human chromosome 17, with smaller parts syntenic to 2 and 22. Human chromosome 17 is associated with the congenital neuropathies Charcot-Marie-Tooth (CMT) disease and Smith-Magenis Syndrome (SMS). I will present data on the analysis of chromosome 11, with the emphasis on a detailed comparison of the CMT and SMS regions in mouse and human. Most likely a reflection of the instability of the region in human (both CMT and SMS are caused by chromosomal deletions), the region is highly re-arranged between the two species. At least nine blocks of contiguous orthology totaling around 8 Mb have been shuffled into different order and orientation in mouse. Also, the mouse region does not feature the various duplications of groups of genes seen in human.

The annotation of both the mouse and the human chromosome will be viewable in Vega (vega.sanger.ac.uk).

Genome Sequencing and Comparative Analysis**Oral Presentation****Saturday November 5****4.15pm – 4.30pm****O-6****ESSENTIAL GENES IN THE MOUSE DEDUCED FROM GENETIC SCREENS**K Hentges¹, B Liu², H Nakamura², M Justice²¹ University of Manchester, Manchester, United Kingdom, ² Baylor College of Medicine, Houston, Texas, United States

We have performed two mutagenesis screens for lethal phenotypes using balancer chromosomes. One screen was localized to mouse chromosome 4, between the STS markers D4Mit281 and D4Mit51. The second screen covered the region between *Trp53* and *Wnt3* on mouse chromosome 11. These screens identified all lethal mutations in the balancer regions, without bias towards any phenotype or stage of death. We have isolated 19 lethal lines on mouse chromosome 4, and 59 lethal lines on chromosome 11, many of which are distinct from previous mutants that map to these regions of the genome. We have characterized the mutant lines to determine the time of death and mutant phenotype. Many of the mutants isolated in both screens have cardiovascular defects. We also performed a pair-wise complementation cross to determine if the mutations are allelic. Assuming a Poisson distribution, we calculated the number of essential genes in each region based on allele frequencies. The percentage of essential genes differs between the balancer regions in our study, and is as high as 44% for the chromosome 11 balancer region, indicating that gene functions are not evenly distributed along the genome. An analysis of genetic conservation across species indicates that this high number of essential genes can be correlated with a high degree of linkage conservation throughout evolution. This initial group of mutants provides a functional annotation of mouse chromosomes 4 and 11, and indicates that many novel developmental phenotypes can be quickly isolated in defined genomic intervals through balancer chromosome mutagenesis screens.

Bioinformatics

Oral Presentation

Saturday November 5

4.30pm – 4.45pm

O-7

EVOLUTIONARY-BASED BIOINFORMATICS ANALYSIS OF PRESYNAPTIC GENESD D. Hadley^{1,2}, T K Murphy², O Valladares², L Ungar^{1,4}, J Kim^{1,4,5}, M Bucan^{1,2,3}Penn Center for Bioinformatics / Genomics and Computational Biology Graduate Group¹, Department of Genetics², School of Medicine³, Department of Computer & Information Sciences / School of Engineering and Applied Sciences⁴, Department of Biology / School of Applied Sciences⁵; University of Pennsylvania, Philadelphia PA 19104

To facilitate identification of *cis*-regulatory elements involved in the transcriptional and translational control of gene expression in the neuronal synapse, we initiated a large-scale comparative analysis of genes implicated in presynaptic function. Although annotation of both protein- and non-protein-coding annotation is available through a number of public databases, such datasets are highly automated and somewhat limited in resolution. Thus, we sought to complement these genome-wide efforts by focusing on 130 presynaptic genes (63Mb), and providing highly curated, in-depth annotation of their genomic neighborhoods. Evolutionary analysis combined with bioinformatics approaches, represent a powerful mechanism for understanding the genomic landscape, and we have employed such methods here. By first focusing on regions undergoing purifying selection and then determining various measures of biological importance *in silico*, we prioritize genomic elements for both *in vitro* and *in vivo* verification. In particular, computational approaches include determining the rate of protein evolution, estimating codon bias on coding sequences, and calculating the folding energy of noncoding elements. In this paradigm, we have annotated novel transcripts, missed exons, candidate miRNAs and putative miRNA targets. In addition to considering genes individually, we also consider them in the context of their gene family where applicable. In so doing, we are beginning to explain diverse gene ontologies, gene expressions and other readily available phenotypes from an evolutionary perspective.

Bioinformatics

Oral Presentation

Saturday November 5

4.45pm – 5.00pm

O-8

INTELLIGENT INFERENCES IN THE OMIC SPACE: TOWARD HIGH-THROUGHPUT *IN SILICO* POSITIONAL CLONING

N Kobayashi, Y Mochizuki, Y Hasegawa, N Heida, K Player, T Toyoda
Genomic Sciences Center, RIKEN, Yokohama, Japan

Since whole genome sequences were first elucidated, knowledge-based ranking of candidate genes has become one of the most important bioinformatics tasks in the forward-genetics and positional-cloning approaches to identify phenotype-responsible gene mutations. This task requires creating a form of artificial intelligence that solves a genetics researcher's problem by learning computationally a vast amount of information elucidated from data ranging from genomic to phenomic levels.

In order to integrate various *omic* annotations and interactions in the databases, we have been applying the coordinate-based integration methodology that we proposed previously [1], rather than the conventional identifier-based integration. The advantage of coordinate-based integration is that it can unite different types of data items having complex many-to-many correspondences that are difficult to represent by identifier-based referencing. Our method is especially effective for integration of distributed databases, since we can now share common genomic sequences to realize the integration of world-wide distributed data based on consistent positions on the same coordinate axes.

By adopting our methodology, we have developed a system that suggests highly promising candidate genes in a given chromosomal interval. The system employs a full-text search engine against biological literature (the Medline abstracts) and handles other *omic* knowledge accumulated in our databases. In the case where few candidates are found by direct keyword search, the system automatically proceeds to infer other candidates through biological networks. Another improvement is that we have added original contextual words to the gene-name dictionary so as to improve the accuracy of gene-name recognition from the literature.

[1] Toyoda, T. and Wada, A. (2004) *Bioinformatics* 20, 1759-65.

Bioinformatics

Oral Presentation

Saturday November 5

5.00pm – 5.15pm

O-9

EMAGE – A SPATIAL DATABASE OF GENE EXPRESSION PATTERNS IN THE DEVELOPING MOUSE EMBRYO. TOWARDS A TOOL FOR COMPUTATIONAL IDENTIFICATION OF SETS OF CO-EXPRESSED GENES FROM IN SITU EXPERIMENTS

J.Christiansen, L Richardson, S Venkataraman, P Stevenson, N Burton, Y Yang, C Semple, R Baldock, D Davidson
MRC Human Genetics Unit, Edinburgh, United Kingdom

EMAGE is a database of spatially mapped in situ gene expression patterns in the developing mouse embryo.

All EMAGE data is housed in a standard framework: the EMAP Digital Atlas of Mouse Development. This consists of at least one representative 3D digital embryo model at most Theiler stages (TS) as well as a standardised nomenclature for the anatomical structures that are present at every TS of development. The digital embryo models are 3D objects and virtual sections can be cut in any plane to reveal internal anatomical detail.

Raw incoming data images are converted to digital representations and mapped spatially into the corresponding regions within the embryo models. At TS07-14 the 3D standard models have anatomical regions defined within them and data spatially mapped into these models is automatically annotated to the corresponding text terms for these structures. This is accompanied by further manual text annotation.

Current data searching can be done spatially by defining a region of interest in a particular embryo model, or by using text terms to find genes expressed within the region and/or named structure. We show in pilot studies that the database can also be used to identify groups of co-expressed genes by hierarchical clustering and domain intersection analyses.

Free software to search EMAGE can be downloaded from <http://genex.hgu.mrc.ac.uk>. The same software can also be used to prepare private databases for in-lab data management or to prepare electronic submissions to EMAGE. Alternatively, specimens can be sent directly to EMAGE for entry into the public database.

Bioinformatics**Oral Presentation****Saturday November 5****5.15pm – 5.30pm****O-10****BEYOND THE DATA DELUGE: ONTOLOGIES FOR COMPLEX BIOLOGICAL SYSTEMS**J A Blake, H J Drabkin, J A Kadin, L Ni, JE Richardson, M E Dolan, A Diehl, D P Hill

The Jackson Laboratory, Bar Harbor, United States

The Gene Ontology is a structured vocabulary system that provides ontologies for functional annotations of all organisms. The GO system enables functional data analysis of very large data sets, e.g., the analysis of micro-array results. The GO annotations for mammalian genomes such as mouse, rat and human are comprehensive. The GO is an open source project; all ontologies and annotations and analysis tools are available at <http://www.geneontology.org/>

MGI is one of the founding groups of the GO and actively involved in the development and implementation of bio-ontologies. One of the major challenges at MGI is to create a human-digestible representation of the wealth of information in our database resource. To this end, we have development an ontology browser, a graphical representation of mouse/human/rat annotation sets for orthologous genes, and a textual representation of the structured annotations.

We are now exploring the power of disease-centric ontologies to support comparative analysis of mouse models and human disease presentations. This work draws on foundational ontologies such as the Gene Ontology (GO), the Anatomical Dictionary for Mouse, and the Mammalian Phenotype Ontology as well as other semantic standards incorporated into the Mouse Genome Informatics (MGI) system.

The Mouse Genome Informatics (MGI) Resource provides information about the genetics, and biology of the laboratory mouse. Community semantic standards facilitate the integration and recovery of mouse information, and the interconnection of mouse data with other biological information.

This work is supported by program project grant HG00330 and grant HG002273 from NHGRI, and grant HD33745 from NICHD.

Models of Human Disease

Oral Presentation

Sunday November 6

9.00am – 9.15am

O-11

GERMLINE TRANSMISSION OF HUMAN CHROMOSOME 21 IN AN ANEUPLOID MOUSE STRAIN WITH DOWN SYNDROME PHENOTYPES

A O Doherty¹, S Ruff², C Mulligan³, M Errington², S Cooke², P Sharpe⁴, S Brandner¹, T Bliss², D Nizetic³, V L J Tybulewicz², E M C Fisher¹

¹ Institute of Neurology - UCL, London, United Kingdom, ² MRC National Institute for Medical Research, London, United Kingdom, ³ Barts - QMW, London, United Kingdom, ⁴ Dental Institute Kings College, London, United Kingdom

At least 5% of all human pregnancies are aneuploid, and ~1 in 700 children are born with Down syndrome (DS) which results from having three copies of human chromosome 21 (Hsa21). DS is the most common known genetic cause of mental retardation and also results in increased susceptibility for other disorders, including developmental deficits. It is a complex disorder that involves multiple Hsa21 genes in interaction with the rest of the genome. To gain insight into the biology of DS, we have generated a new type of mouse model in which an almost complete human chromosome, Hsa21, segregates through the germline. We present evidence that this trans-species aneuploid mouse strain, 'Tc1', displays phenotypic alterations in behaviour, synaptic plasticity, cerebellar neuronal number and heart development that relate directly to human DS and to other partial trisomy models of DS. Transchromosomic mouse lines such as Tc1 may represent useful genetic tools to dissect other aneuploidies and complex human genetic conditions.

Models of Human Disease**Oral Presentation****Sunday November 6****9.15am – 9.30am****O-12****CELL-AUTONOMOUS MITOGENESIS RESPONSE DEFECT TO HEDGEHOG SIGNALING IN DOWN SYNDROME MICE**

R J Roper, L B Baxter, N G Saran, D K Klinedinst, P A Beachy, [R H Reeves](#)
Johns Hopkins University School of Medicine, Baltimore, MD, United States

Ts65Dn mice are trisomic for orthologs of about half of the genes on human chromosome 21 and display a number of developmental anomalies analogous to those in Down syndrome. In particular, we showed that alterations in the cerebellum of trisomic mice mimic the pathology of Down syndrome, including a reduced number and density of granule cell neurons in Ts65Dn mice which correctly predicted a corresponding phenotype in humans with Down syndrome. We have traced the granule cell deficit to the earliest point at which trisomic and euploid cerebella diverge, identified granule cell precursors (gcp) as the affected cells and shown that the response of gcp to the effects of sonic hedgehog (Shh) growth factor-induced mitogenic pathway underlies inadequate generation of granule cells. Trisomic gcp have an intrinsic deficit in response to Shh, showing a reduced but dose-dependent response to Shh protein *in vitro*. These results suggested that increasing local Shh concentrations might overcome all or part of this deficit *in vivo*. Systemic treatment of newborn trisomic mice with a small molecule agonist of Hedgehog pathway activity increased mitosis and restored granule cell precursor populations in the critical developmental period immediately after birth. This is the first report of amelioration of a neuronal deficit in Down syndrome, and identifies a target for possible clinical interventions in a central component of cognitive disability in trisomy 21.

Models of Human Disease**Oral Presentation****Sunday November 6****9.30am – 9.45am****O-13****ENU MUTAGENESIS AND THE PHENOTYPE-DRIVEN APPROACH: IS IT WORTH THE EFFORT?**K L Svenson, B Paigen, L L Peters

The Jackson Laboratory, Bar Harbor, Maine, United States

Several large-scale mouse mutagenesis programs have been established in the last decade in an effort to accelerate our understanding of gene function and ultimately lead us to better management of human disease. These programs are largely based on strategies developed in other model organisms such *C. elegans*, *D. rerio*, and *D. melanogaster*. In translating this approach to the mouse, an enormous commitment of resources is required. We report our progress in the Heart, Lung, Blood and Sleep Mutagenesis Program at The Jackson Laboratory, initiated in late 2000. We developed novel, high-throughput, robust primary screens designed specifically for mice that have been validated by more invasive means and by other laboratories. We have established over 60 heritable mutants and have identified new alleles and functions for known genes. Many other models are currently in heritability testing. We have mapped a subset of our new mutants and will report on the varied success in mapping including resultant QTLs when genetic backgrounds are mixed. Our diverse protocol is easily extended to screening existing knockout, transgenic and inbred strains. We have screened 43 inbred strains using our protocol, providing valuable phenotype information for choosing strains to use for mapping. Whole-animal mutagenesis followed by high throughput phenotyping is clearly an effective, unbiased tool for identifying genes underlying human disease.

Models of Human Disease**Oral Presentation****Sunday November 6****9.45am – 10.00am****O-14****REVERSE GENETICS BY ENU-BASED GENE-DRIVEN MUTAGENESIS IN THE MOUSE**

Y Sakuraba, H Sezutsu, K R Takahasi, Y Nakai, M Uchiyama, R Fukumura, T Murata, H Kaneda, S Wakana, T Noda, T Shiroishi, Y Gondo
RIKEN Genomic Sciences Center, Yokohama, Japan

ENU mutagenesis has been widely used to generate batteries of human disease models. It primarily aims to establish mutant strains by phenotype-driven approach, in particular, to identify yet unknown responsible genes for human diseases. One of the major objectives of the RIKEN mouse mutagenesis project is to identify mouse models for human common diseases. To securely retain useful mutant strains, all the G1 sperm were subjected to cryopreservation. Currently, about 10,000 G1 males have been subjected to the sperm cryopreservation. The G1 frozen sperm archive also provides a mutant mouse library for reverse genetics. To make this gene-driven mutagenesis feasible, 1) corresponding G1 genomic DNA archive construction, 2) PCR primer designing for target genes, 3) high throughput point mutation discovery system and 4) live mouse retrieval are necessary. Currently, we set a genomic DNA archive for ~8,000 G1 mice. We have listed 195 target genes and designed their PCR primers. We firstly used the direct sequencing method to detect ENU-induced mutations and then added Temperature Gradient Capillary

Electrophoresis as a quick pre-screening system. We have identified more than 200 ENU-induced mutations. The molecular characterization revealed the nature of ENU mutagenesis in the mouse genome. For instance, we have identified even a minor fraction of the clonal expansion of a common ancestor mutation in the G0 spermatogenesis. Finally, we have retrieved more than 30 live mouse strains out of ~200 identified mutations from the archive. Some G3 offspring have already been born in several strains and subjected to phenotype screening.

Models of Human Disease**Oral Presentation****Sunday November 6****2.00pm – 2.15pm****O-15****A SENSITIZED ENU MUTAGENESIS SCREEN FOR GENETIC MODIFIERS OF RHEUMATOID ARTHRITIS AND INFLAMMATORY BOWEL DISEASE**E Douni, E Makrinou, G Mermelekas, N Giannakas, G Kollias

Institute of Immunology, Biomedical Sciences Research Center, Alexander Fleming, Athens, Greece

Genome-wide, random mutagenesis with the ethylating mutagen N-ethyl-N-nitrosourea (ENU) has become an attractive method to track the role of virtually any gene in a particular phenotype. In particular, ENU mutagenesis of disease sensitized animals offers unique opportunities to discover gene functions directly associated with prevention or therapy of diseases. We have thus initiated a Program of sensitized ENU mutagenesis screen applied on our established TNF^{ΔARE} model of arthritis and Crohn's-like inflammatory bowel disease (IBD), to identify modifier gene candidates associated with development of these diseases. By using simple and accurate phenotypic screens, clinical score for arthritis and macroscopic or histological analysis for IBD, we are selecting the individual progeny that display disease attenuation. By screening 4304 G3 offspring we have identified 3 families which show significant delay on the onset and progression of arthritis and 2 families with dramatic attenuation of IBD. In parallel to the sensitized screens we have also identified novel recessive phenotypes ie. a mouse mutant model which shows severe osteopetrosis, defect in tooth eruption and complete lack of osteoclasts.

A genetic mapping approach is currently in progress which involves an F2 intercross mapping scheme and genome-wide microsatellite typing, in order to identify potential susceptibility loci. Initial mapping efforts have already identified candidate chromosomal regions for specific mutants, whereas fine mapping using SNPs analysis is currently underway. Once identified these novel gene functions may constitute validated pharmaceutical targets for the treatment of chronic inflammatory disease.

Models of Human Disease

Oral Presentation

Sunday November 6

2.15pm – 2.30pm

O-16

DOMINANT MUTATIONS OF COL4A1 RESULT IN BASEMENT MEMBRANE DEFECTS WHICH LEAD TO ANTERIOR SEGMENT DYSGENESIS AND GLOMERULOPATHY

T Van Agtmael¹, U Schlötzer-Schrehardt³, L McKie², DG Brownstein⁴, J J Mullins¹, E Pöschl⁵, I J Jackson¹

¹ Molecular Physiology, Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, United Kingdom, ² Medical Research Council Human Genetics Unit, Edinburgh, United Kingdom, ³ Department of Ophthalmology, University of Erlangen-Nürnberg, Erlangen, Germany, ⁴ Division of Pathology, School of Molecular and Clinical Medicine, University of Edinburgh, Edinburgh, United Kingdom, ⁵ Department of Experimental Medicine I, University of Erlangen-Nürnberg, Erlangen, United Kingdom

Members of the type IV collagen family are essential components of all basement membranes and define structural stability as well as tissue-specific functions. The major isoform, $\alpha 1(\text{IV})$, contributes to the formation of many basement membranes and its deficiency causes embryonic lethality in mouse. We have identified an allelic series of three ENU induced dominant mouse mutants with missense mutations in the gene *Col4a1* encoding the $\alpha 1(\text{IV})$ subunit chain. Two severe alleles (*Bru* and *Svc*) have mutations affecting the conserved glycine residues in the Gly-Xaa-Yaa collagen repeat. *Bru* heterozygous mice display defects similar to Axenfeld-Rieger anomaly including iris defects, corneal opacity, vacuolar cataracts, significant iris/corneal adhesions, buphthalmos and optic nerve cupping, a sign indicative of glaucoma. Kidneys of *Bru* mice have peripheral glomerulopathy characterised by hypertrophy and hyperplasia of the parietal epithelium of Bowman's capsule. A milder allele (*Raw*) contains a mutation in the Yaa residue of the collagen repeat and was identified by a silvery appearance of the retinal arterioles. All phenotypes are associated with basement membrane defects that affect the eye, kidney and other tissues. This allelic series shows that mutations affecting the collagen domain cause dominant negative effects on the expression and function of the major collagen IV isoforms $\alpha 1(\text{IV})$ and pathological effects vary with the individual mutations.

Models of Human Disease

Oral Presentation

Sunday November 6

2.30pm – 2.45pm

O-17

ALLELIC SERIES IN THE MC4R GENE DEMONSTRATES THAT VARYING LEVELS OF OBESITY CORRELATES WITH RECEPTOR FUNCTIONT P Meehan¹, K Tabeta², B Beutler², M J Justice¹¹ Baylor College of Medicine, Houston, TX, United States, ² The Scripps Research Institute, La Jolla, CA, United States

Defects in the melanocortin 4 receptor (*Mc4R*) have a well-established role in obesity in both humans and mice. Herein, we report the isolation of an allelic series through ENU mutagenesis in the mouse *Mc4R* gene that mimics the obesity found in human patients. As in humans, the severity of the obesity phenotype is directly related to the amount of function remaining for each receptor. One missense mutation, South Beach, fails to translocate to the surface of the cell in *in vitro* assays and, therefore, has no receptor activity. A second missense receptor mutation, Fat Boy, has signaling properties similar to the wild type Mc4R even though it has only 14% of the surface expression levels as measured by specific binding. Both mutant mice display obesity although the South Beach mice are significantly heavier. Similar to the *Mc4R* knockout mice and humans carrying *Mc4R* mutations, mice heterozygous for the mutations described here display an intermediate level of obesity as compared to control littermates. This effect demonstrates the sensitivity of this receptor such that partial haploinsufficiency still yields a discernable phenotype. These mutant mice will serve as good models for the variation in obesity found in humans with mutations in Mc4R.

Models of Human Disease

Oral Presentation

Sunday November 6

2.45pm – 3.00pm

O-18

MUTANT MOUSE MODELS OF NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE SHOW DEFECTS IN INSULIN SECRETION AND GLUCOSE TOLERANCEH C Freeman¹, K Shimomura², E Horner¹, F M Ashcroft², R D Cox¹¹ Medical Research Council, Harwell, United Kingdom, ² Oxford University, Oxford, United Kingdom

Insulin release from pancreatic beta-cells is regulated by glucose metabolism. When plasma glucose levels rise, intracellular ATP levels increase, and close ATP-sensitive potassium (K_{ATP}) channels. This results in membrane depolarisation, activation of voltage-gated Ca^{2+} channels, influx of Ca^{2+} and exocytosis of insulin-containing vesicles.

The C57BL/6J mouse displays glucose intolerance and reduced insulin secretion. QTL mapping identified Nicotinamide Nucleotide Transhydrogenase (*Nnt*) as a strong candidate gene. *Nnt* is a nuclear-encoded mitochondrial protein thought to be involved in free radical detoxification.

We identified two ENU-induced point mutations in *Nnt* (N68K, G745D). *Nnt* mutant mice were glucose intolerant and secreted less insulin during a glucose tolerance test. Isolated islets also showed impaired insulin secretion in response to glucose, but not to tolbutamide. This was a consequence of reduced ATP generation at elevated glucose in *Nnt* mutant islets. We also used siRNA to knock down *Nnt* in the insulin-secreting cell line MIN6. This resulted in a dramatic reduction in insulin secretion and in the rise in $[Ca^{2+}]_i$ evoked by glucose, but not elicited by the sulphonylurea tolbutamide. Both applications therefore confirmed the functional role of *Nnt* in insulin release.

We hypothesise that *Nnt* mutations impair beta-cell mitochondrial metabolism which thereby accounts for the lower ATP production, and enhances the activity of K_{ATP} channels. Consequently, glucose-dependent beta-cell electrical activity and insulin secretion are impaired. This in turn leads to impaired glucose tolerance in the animal.

Models of Human Disease**Oral Presentation****Sunday November 6****3.00pm – 3.15pm****O-19****FILAMIN B MUTATIONS CAUSE CHONDROCYTE HYPERTROPHY DURING SKELETAL DEVELOPMENT**MJ Justice, L Zheng

Baylor College of Medicine, Houston, Texas, United States

Mutations in *Filamin B*, a gene encoding a cytoplasmic actin binding protein, have been found in five human skeletal disorders: boomerang dysplasia, spondylocarpotarsal syndrome, atelosteogenesis I, atelosteogenesis III and Larsen syndrome. To study the role of Filamin B in mammalian skeletal development, we generated mice using an ES cell line trapped with a β -galactosidase-neomycin resistance fusion gene. The mice lacking the full-length protein display ectopic ossification of various bone elements, most prominently in the cervical and thoracic vertebrae, sterna, chondracostal cartilage and carpal bones. The phenotypic abnormalities mimic those of the human skeletal disorders with nonsense mutations in *Filamin B* gene. The aberrant bone formation is due to ectopic chondrocyte hypertrophy, which can also be caused by loss of histone deacetylase 4 or constitutive expression of Runx2 in chondrocytes. Together with our previous finding that filamin B may interact with *Odz4*, a transmembrane receptor implicated in skeletogenesis, these results suggest that Filamin B mediates a signaling cascade controlling chondrocyte hypertrophy during bone development.

Models of Human Disease

Oral Presentation

Sunday November 6

3.15pm – 3.30pm

O-20

GENETIC INTERACTION BETWEEN *ITCH* AND *NOTCH1* IN A MOUSE AUTOIMMUNE DISEASE MODEL SUGGESTS A ROLE FOR *NOTCH1* SIGNALLING IN NEGATIVE SELECTION AND CELL SURVIVALL E Matesic¹, D C Haines², N G Copeland¹, [N A Jenkins](#)¹¹ National Cancer Institute, Frederick, MD, United States, ² SAIC-Frederick, Frederick, MD, United States

Itch represents one of the few single gene mouse autoimmune disease models. Homozygous *itchy* (*itch*) mice develop a progressive disease characterized by systemic inflammation that proves fatal at 6-8 months of age due to congestive pneumonia. This autoimmune-like disease results from a loss of function mutation in a HECT E3 ubiquitin protein ligase. Phylogentic and *in vitro* analyses suggest that *Itch* is a negative regulator of Notch signalling. To assess the biological role of *Itch* in Notch signalling, we bred *itch* mice to mice carrying an activated *Notch1* transgene under the control of the *lck* proximal promoter. Interestingly, *itch* mice that carry the *Notch1* transgene are significantly smaller than their littermates and die by 12 weeks of age. They also have the same autoimmune disease seen in *itch* animals; however, the disease is much more severe and develops much sooner. T cell development is also perturbed in these mice, with a reduction in the number of CD4, CD8 double positive cells and an increase in the number of double negative and single positive cells. TUNEL staining shows reduced apoptosis in the thymus of *itch* + *Notch1* transgenic animals and antibody staining for Notch1 displayed increased levels of full length (FL) Notch1 in the thymus but not in the spleen. Collectively, these results demonstrate that *Itch* and *Notch1* synergize in this autoimmune-like disease, and suggest that increased Notch1 signalling through increased FL Notch1 in thymocytes provides a survival signal to cells that were otherwise fated to die after negative selection.

O-21**DISCOVERY OF GENES AND PATHWAYS ASSOCIATED WITH DISEASE BY THE MOUSE ENU-MUTAGENESIS APPROACH**

C P D Tu, C Chang, L Y Chau, J J Chen, C F Cheng, Y Chern, B C Shyu, J Y Wu, J J Y Yen, Y T Chen
IBMS, Academia Sinica, Taipei, Taiwan

We, as a team of physicians (cardiologists, neurologists, metabolic specialists), biomedical scientists, and human and molecular geneticists, established a comprehensive mouse clinic in which highly sophisticated and specialized screening tests for disease phenotypes can be performed. The overall goals of our programs are to unravel genes and pathways associated with human diseases and to generate mouse models for them.

Using a genome-wide mutagenesis approach with *N*-ethyl-*N*-nitrosourea (ENU) to produce mutant mice, followed by phenotype screening and high-throughput genotyping to localize the mutations, we have identified mouse models of neurological diseases (brain atrophy, necrotizing encephalopathy, hydrocephalus, pain hyper-sensitivity, pentobarbital resistance), cardiovascular diseases (aortic stenosis, cardiac arrhythmia), renal disease (hydronephrosis), hematology (lymphopenia) and metabolic diseases (human maple syrup urine-like disease and fatty acid oxidation defects). These mouse models are unique, as most of them have not been reported from ENU programs elsewhere. We have confirmed the heritability and performed whole genome homozygosity mapping (MassARRAY SNP genotyping platform) using a panel of 299 SNPs on most of these models.

In the cases of the mouse models resembling human maple syrup urine disease, fatty acid oxidation defects, and lymphopenia, the responsible genes causing the diseases have been identified. We will continue to identify more mouse models with distinct phenotypes. Insights obtained from characterizing novel genes and pathways of human diseases and the mouse models generated will not only enhance our understanding of the patho-physiology of the diseases but also improve the diagnosis and facilitate the development of more effective therapies.

Stem Cells

Oral Presentation

Monday November 7

9.00am – 9.15am

O-22

INSERTIONAL MUTAGENESIS IDENTIFIES GENES THAT PROMOTE THE IMMORTALIZATION OF HEMATOPOIETIC STEM AND PROGENITOR CELLSY Du, N A Jenkins, [N G Copeland](#)

National Cancer Institute, Frederick, MD, United States

Retroviruses induce hematopoietic disease via insertional mutagenesis of cancer genes and provide valuable molecular tags for cancer gene discovery. Recently, we found that insertional mutagenesis can also identify genes that promote the immortalization of hematopoietic stem as well as hematopoietic progenitor cells; cells which normally have only limited self-renewal. Transduction of primary mouse bone marrow cells with replication-incompetent murine stem cell virus (MSCV) expressing only a *neo* marker gene, followed by serial passage of the infected cells in liquid culture containing SCF and IL-3, selects for immortalized immature myeloid cells with neutrophil and macrophage differentiation potential. In contrast, immortalized cells that are blocked at the early hematopoietic /stem cell stage can be selected by growing the infected cells in SCF plus FLT3L. Cloning and sequence analysis of the MSCV integration sites in the immortalized lines showed that in the majority of cases, immortalization resulted from the insertional mutagenesis of known leukemia genes or genes that are thought to regulate them. Thus, these immortalization genes by their nature could also be involved in the immortalization of the leukemic stem cell and represent attractive drug targets for treating cancer. Since our genetic screen employs a replication defective virus, it is not limited to murine cells. The MSCV LTR is also active in human cells. By using amphotropic packaging cells it may therefore also be possible to perform these screens directly in human cells. Finally, this screen could also theoretically be used to identify immortalization genes for many other types of stem/progenitor cells.

Stem Cells

Oral Presentation

Monday November 7

9.15am – 9.30am

O-23

A SENSITIZED MOUSE MUTAGENESIS SCREEN FOR NOVEL LOCI REGULATING MAMMALIAN NEURAL CREST DEVELOPMENT

D Watkins-Chow, D Silver, S K Loftus, I Matera, D Larson, C Rivas, E Elliot, [W J Pavan](#)
Genetic Disease Research Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, United States

A mouse mutagenesis program is in progress to screen for mutations disrupting mammalian neural crest cell development and to develop an archived resource of mutagenized mice. The focused phenotype screen has been designed to detect mutations specifically affecting melanocyte and peripheral nervous system development. Progeny of N-ethyl-N-nitrosourea (ENU) treated mice are being bred to *Sox10^{LacZ}/+* mice carrying a disruption in a transcription factor important for neural crest cell development. These mice manifest subclinical neural crest defects due to haploinsufficiency for SOX10. The sensitized screen uncovers mutations that act synergistically with *Sox10^{LacZ}/+* resulting in clinically visible phenotypes such as white coat color spotting. Additionally, third generation embryos are being generated in a backcross to screen for embryonic phenotypes that alter expression of the LacZ reporter gene in Sox10 expressing cells. Our 3-generation breeding strategy utilizes two different mouse strains, BALB/cJ and C57BL/6J, to facilitate mapping of both dominant and recessive phenotypes and allows for subsequent recovery of lethal phenotypes. To date, we have identified two heritable phenotypes from the dominant screen of 100 pedigrees and five heritable mutations from a recessive embryonic screen of 60 pedigrees. The phenotypes observed in the embryonic screen include ectopic expression of Sox10-LacZ, abnormal glial cell patterning and migration, and loss of Sox10-LacZ expression in subsets of peripheral nervous system cell lineages. None of the loci localize to genes for the major mouse spotting mutants, demonstrating the feasibility of this approach for identifying novel loci regulating neural crest development.

Stem Cells

Oral Presentation

Monday November 7

9.30am – 9.45am

O-24

A MUTATION IN STRATIFIN (14-3-3 SIGMA) IS PRESENT IN REPEATED EPILATION MICEL D Siracusa², R A Liddell³, A Parker¹, J Kinne¹, B J Herron¹¹ Genomics Institute, Wadsworth Center, Troy, NY, United States, ² Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, PA, United States, ³ Cancer Diagnosis Program, NCI, Rockville, MD, United States

The mouse repeated epilation (*Er*) mutation causes a semidominant phenotype that results in the cyclic loss of hair and an increased incidence of skin cancers. Homozygous *Er/Er* embryos have profound defects in epidermal development and keratinocyte differentiation that result in neonatal lethality. We have generated a high-resolution genetic map that localized the *Er* mutation to an 800 kilobase interval on mouse Chromosome 4. This interval included Stratifin (*sfn*), a member of the 14-3-3 protein family. Stratifin is repressed in several human cancers and is highly expressed in differentiating epidermis. We identified an insertion mutation in *sfn* that produces a truncated *sfn* protein. This truncated *sfn* is expressed in *Er/+* mice and may act as a dominant negative molecule. Confirming the identity of *Er* as *sfn*, we were able to rescue the *Er/+* phenotype with a BAC carrying the normal *sfn* protein. Ongoing work will address the functional nature of this mutation, and elaborate the molecular mechanisms that cause the *Er* phenotype.

Stem Cells

Oral Presentation

Monday November 7

9.45am – 10.00am

O-25

MECHANISMS OF PHENOTYPIC VARIABILITY IN ADRENOCORTICAL DYSPLASIA (*ACD*) MICEJ E Hutz, A Belcher, C E Keegan

University of Michigan, Ann Arbor, MI, United States

Adrenocortical dysplasia (*acd*) is a spontaneous recessive mouse mutation that originated on the DW/J strain. *acd* mutant mice exhibit a strain-dependent pleiotropic phenotype; on the DW/J strain, the phenotype is lethal shortly following birth and includes caudal truncation, vertebral segmentation, and limb patterning defects. In contrast, *acd* mutant mice on a mixed DW/J X CAST/Ei background have developmental defects in urogenital ridge structures and can survive into adulthood, suggesting that one or more modifier genes influences the *acd* phenotype. We have previously characterized the *acd* mutation as a splicing defect in a gene (*Acd*) that encodes a novel component of the complex of telomere binding proteins that functions to maintain telomere integrity. Here, we report widespread expression of *Acd* mRNA in mouse embryos. We observed increased expression in the limb buds and developing tail, which are regions of highly proliferative cells that correspond to the structural defects observed in *acd* mutant embryos. In addition, using a highly sensitive fluorescent RT-PCR assay in *acd* mutant mouse embryonic fibroblasts, we can detect a very small percentage of appropriately spliced *Acd* mRNA transcripts from the mutant allele. To identify candidate modifier loci, we genotyped 11 DW/J^{*acd*} X CAST/Ei F₂ mutant mice with a panel of 402 SNPs. The SNP genotyping data so far are inconclusive. Our studies suggest the possibility that *acd* is a hypomorphic allele and indicate that further studies will be required to localize modifier genes.

Development

Oral Presentation

Monday November 7

11.00am – 11.15am

O-26

WHIRLIN COMPLEXES WITH P55 AT THE STEREOCILIA TIP DURING HAIR CELL DEVELOPMENTP Mburu¹, Y Kikkawa², R Romero¹, S Townsend¹, H Yonekawa², S D M Brown¹¹ MRC Mammalian Genetics Unit, Harwell, Oxon, United Kingdom, ² Tokyo Metropolitan Institute of Medical Science (Rinshoken), Tokyo, Japan

Actin cytoskeleton remodelling is fundamental to a variety of cellular processes including morphological alterations at the cell surface. Hearing in mammals is dependent upon the proper development of actin-filled stereocilia at the surface of hair cells in the inner ear. Recent work has established that whirlin, a PDZ protein, localises to the stereocilia tips and by virtue of mutations in the whirlin gene has been shown to play an important role in stereocilia development. Myosin XVa interacts with whirlin and is responsible for localising whirlin at the stereocilia tip. We have previously demonstrated that whirlin shows an extraordinary expression pattern that traverses the stereocilia bundle during its development. We now show using yeast 2-hybrid screening, in vitro and in vivo pull-down assays that whirlin interacts with the MAGUK protein p55. p55 is known in erythrocytes to form a trimeric complex with protein 4.1R and the transmembrane protein glycophorin C promoting actin cytoskeleton assembly. We find that both p55 and protein 4.1R are expressed in both the developing stereocilia bundle and in the shorter microvilli-like stereocilia surrounding the graded stereocilia. In the whirler mutant, expression of p55 and protein 4.1R in hair cell stereocilia fades out prematurely from around P5. In the shaker2 (myosin XVA) mutant, expression of both p55 and 4.1R is completely abolished. We propose that whirlin forms a complex with p55 and protein 4.1R at the stereocilia tip that mediates actin polymerisation in response to some as yet unidentified external signal.

Development**Oral Presentation****Monday November 7****11.15am – 11.30am****O-27****THE NOTCH LIGAND DELTA1 INTERACTS WITH PDZ-DOMAIN CONTAINING PROTEINS**C Hoefler, S Pfister, J Adamski, G Przemeck, M Hrabé de Angelis

GSF-National Research Center, Institute of Experimental Genetics, Neuherberg/Munich, Germany

Cell-cell signaling by the Notch-pathway is mediated by the interaction of the transmembrane receptor Notch with its ligands Delta and Serrate (Jagged in vertebrates) presented on adjacent cells. Whereas signal transduction to Notch expressing cells has been described, it is yet unclear whether Delta-dependent signaling may also exist within the Delta expressing cell. Recently, we report on the identification of proteins interacting with the intracellular domain of mouse Delta1 (Dll1cyto). Among others, we identified several PDZ-domain containing proteins as Dll1cyto interacting (Pfister et al., 2003). For example, the interaction with Magi2 (also known as Acvrin1 or S-SCAM) and Magi3 was confirmed by in vitro and in vivo systems. Interacting domains could be delimited to the fourth PDZ domain of Magi2, the fifth PDZ domain of Magi3 and to the C-terminal PDZ-binding motif of Dll1. In situ expression analyses as well as immunohistochemistry in mouse embryos revealed that Dll1 and newly identified interacting partners show partly overlapping but distinct expression patterns, for example, in the central nervous system. Here, we will present newest results on Dll1-interacting PDZ proteins and discuss possible functions of Dll1cyto-dependent mechanisms.

Literature:

Pfister, S. et al. 2003, JMB 333, 229-235

Development

Oral Presentation

Monday November 7

11.30am – 11.45am

O-28

A MAJOR ENHANCER FOR LIMB-SPECIFIC EXPRESSION OF *SHH* RESIDES IN 1MB UPSTREAM OF THE CODING SEQUENCE, AND HAS BEEN LOST IN LIMBLESS SPECIEST. Sagai¹, M. Hosoya¹, Y. Mizushima¹, H. Masuya², M. Tamura¹, T. Shiroishi¹¹ National Institute of Genetics, Mishima, Shizuoka-ken, Japan, ² RIKEN Genome Sciences Center, Tsukuba Igaragi-ken, Japan

The paired fins of teleost fishes and tetrapod limbs have evolved from a common ancestral appendage. Our previous studies revealed that an intronic sequence of the mouse *Lmbr1*, localized in 1Mb upstream of the *Shh* coding sequence, is highly conserved among tetrapods and teleost fishes, and has a single base substitution in mouse preaxial polydactyly mutants. We examined the physical linkage of *Shh* and the conserved sequence in a teleost fish, medaka. The sequence was found in the intron 5 of the medaka *Lmbr1* homolog, and is placed in the same scaffold as the *Shh* coding. These facts suggest that the conserved sequence is *cis*-acting regulator for limb-specific *Shh* expression, and that the physical linkage of the *Shh* coding and the *cis*-acting regulator evolved prior to divergence of teleost fishes and tetrapods. We intended to directly examine the role of the conserved sequence by targeted mutation to eliminate the sequence in the mouse. The knockout mouse showed a complete loss of *Shh* expression in the limb buds and severe amputation of distal elements of the limbs, resembling *Shh* coding knockout mouse and human congenital deformity named Acheiropodia. Notably, this sequence has been lost in two independent lineages of limbless species, limbless newt and snakes. Thus, the conserved sequence contains a major enhancer for limb-specific *Shh* expression, and is indispensable for the development of distal limb structures. Furthermore, loss of this conserved sequence might be involved in evolution of the limbless species.

Development

Oral Presentation

Monday November 7

11.45am – 12.00pm

O-29

THM1 IS A NOVEL NEGATIVE REGULATOR OF MOUSE SONIC HEDGEHOG (SHH) SIGNALINGP V Tran¹, B J Herron¹, P J Scherz², H Qiu¹, A Turbe-Doan¹, K Parker¹, D R Beier¹¹ Brigham and Women's Hospital, Boston, MA, United States, ² Harvard Medical School, Boston, MA, United States

The SHH signaling pathway plays a fundamental role in mammalian embryonic development. The signaling cascade is triggered by binding of the SHH ligand to the transmembrane receptor, Patched (*Ptc*), which releases its repression of the signal transducer Smoothed (*Smo*). The SHH signal culminates on Glioblastoma (*Gli*) transcriptional regulators which activate target genes. We report the characterization of alien (*aln*), a novel mutant mouse, which displays a phenotype characteristic of inappropriate activation of SHH signaling; *aln* mutants exhibit preaxial polydactyly, craniofacial abnormalities and misexpression of SHH target genes in the neural tube and limb bud. Genetic analyses suggest that the *aln* gene product acts as a negative regulator downstream of *Smo* but upstream of *Gli2*. Using positional cloning, we have identified a missense mutation in an evolutionarily conserved N-terminal amino acid in a novel gene we call *Thm1* (Tetratricopeptide repeat (TPR)-containing Hedgehog modulator 1). We found GLI3 activator: GLI3 repressor ratios to be 10-fold higher in *aln* anterior limb buds relative to wild-type, suggesting a role for *Thm1* in regulating cleavage of the GLI3 transcriptional activator to its repressor form. We are currently investigating *in vitro* whether *Thm1* may have a direct role in this process. The predicted protein structure of the *Thm1* product reveals multiple TPR domains, which are known to enable the assembly of macromolecular complexes. It is possible that *Thm1* may function in the formation of complexes necessary to bring together different components required for GLI3 processing.

Epigenetics, Chromosomes and Chromatin**Oral Presentation****Monday November 7****2.00pm – 2.15pm****O-30****BALANCED EXPRESSION BETWEEN THE X CHROMOSOME AND AUTOSOMES IN GERM CELLS AND IN EARLY MOUSE DEVELOPMENT**D K Nguyen, [C M Disteché](#)

University of Washington, Seattle, United States

Monosomy of the X chromosome due to divergence between the sex chromosomes caused by degeneration of the Y lead to dosage compensation mechanisms to restore balanced expression between the X and the autosomes. In *Drosophila*, up-regulation of the male X achieves dosage compensation. Likewise, we have previously shown that mammals up-regulate their active X chromosome in adult somatic tissues. Together with X inactivation, this mechanism would maintain balanced expression between the X and autosomes and between the sexes. Presently, we have used microarray data to show that the X chromosome is expressed but not up-regulated in spermatids and secondary oocytes, preserving balanced expression of the genome in these haploid cells. Furthermore, similar expression levels between the X chromosome and autosomes were observed as early as the zygote and 2-cell stage; this doubling of global transcription from the X in diploid cells was maintained throughout development. Our results imply that upon fertilization, up-regulation of the active X, probably mediated by either removal or onset of epigenetic modifications, must rapidly occur to achieve the observed dosage compensation.

Epigenetics, Chromosomes and Chromatin

Oral Presentation

Monday November 7

2.15pm – 2.30pm

O-31

A DRAFT METHYLATION MAP AT 4000 *NotI* SITES IN THE C57BL/6J GENOMEH Nagase¹, F Song¹, M T Kimura¹, K Fujiwara¹, E Kitamura², W A Held¹¹ Roswell Park Cancer Institute, Buffalo, NY, United States, ² Nihon University, Tokyo, Japan

DNA methylation is an important epigenetic modification in mammals. In order to understand the significance of DNA methylation, we utilized a method for global DNA methylation analysis using Restriction Landmark Genomic Scanning (RLGS) coupled with Virtual image RLGS software (ViRLGS) (Nucleic Acids Res. 31, 4490-4496, 2003). When using a methylation sensitive enzyme such as *NotI* as the restriction landmark, the comparison between real and *in silico* RLGS profiles of the genome provides a methylation map of genomic *NotI* sites. *Mus musculus* (C57BL/6J) RLGS patterns (*NotI-PstI-PvuII* and *NotI-PvuII-PstI* enzyme combinations using adult testis, brain, colon, kidney, liver, and muscle genomic DNAs) were compared with an *in silico* image pattern estimated from published genome sequences using the Vi-RLGS software. *NotI* sites of RLGS spots which were present in a Vi-RLGS pattern but absent in real RLGS patterns were considered methylated sites due to the influence of 5'-methylcytosine on *NotI* landmark detection. Approximately 1,000 constitutively methylated genomic *NotI* sites, 3,000 constitutively unmethylated sites and 150 tissue-specific methylated sites, have been plotted in the C57BL/6J genome and created a methylation map with a chromosome banding pattern. We will also present the similar methylation status in the human genome confirmed at several conserved CpG islands. The application of a quantitative whole-genome methylation analysis by Vi-RLGS and real RLGS to the mouse genomes provides a novel method for identifying specific differences in DNA methylation associated with alterations in chromatin structure that are associated with important biological phenomena such as differentiation, proliferation, aging, and diseases.

Immunity and Infection

Oral Presentation

Tuesday November 8

9.00am – 9.15am

O-32

GENETIC ANALYSIS OF HOST IMMUNITY TO EXPERIMENTAL INFECTION WITH VIRULENT *MYCOBACTERIUM TUBERCULOSIS* USING MOUSE MODELI Kramnik

Harvard School of Public Health, MA, United States

We have mapped a major genetic locus *sst1* (susceptibility to tuberculosis 1) on mouse chromosome 1, and utilized positional cloning to identify a candidate gene *lpr1*, intracellular pathogen resistance 1, (Pan et al, *Nature* 2005, 434:767). The *lpr1* gene controls macrophage-mediated mechanism of innate immunity to intracellular pathogens *Mycobacterium tuberculosis* (MTB) and *Listeria monocytogenes* (LM). Expression of the full length copy of this gene in the *sst1*-susceptible macrophages increased their ability to control multiplication of intracellular pathogens MTB and LM *in vitro*, prevented necrosis and turned on the apoptotic program of cell death in the infected cells. The *lpr1* gene encodes a predicted nuclear protein that has structural homology with known eukaryotic transcriptional coactivators. The susceptible allele of *sst1* did not confer an overt immunodeficiency, but rather specifically affected progression of lung tuberculosis. Four additional genetic loci contributing to tuberculosis resistance were mapped in crosses involving the *sst1* congenic mice and their genetic interactions with the *sst1* were demonstrated. While the genetic polymorphism within the *sst1* locus is due to *de novo* mutation in C3HeB/FeJ substrain of C3H, some of the novel tuberculosis resistance loci are likely to represent ancestral polymorphisms. No *lpr1* homologues were found in yeast, *C.elegans* and insects. Human homologue of the *lpr1* gene exists and has been associated with susceptibility to tuberculosis in human populations in Africa. Our studies demonstrate that mouse model of infection with MTB is useful for dissecting pathogenesis and genetic control of such complex genetic trait as susceptibility to tuberculosis. Funded by NIH NAIID and NIH HLBI.

O-33

TLR EXPRESSION PATTERNS DURING *T. CONGOLENSE* INFECTION IN MICE

J K Nganga², S J Kemp³, F Iraqi¹

¹ International Livestock Research Institute, Nairobi Nairobi, Kenya, ² Jomo Kenyatta University of Agriculture and Technology, Nairobi Nairobi, Kenya, ³ School of Biological Sciences, University of Liverpool, Liverpool, United Kingdom

Marked differences between inbred strains of mice in their response to trypanosomiasis can be exploited to analyze the genetic basis of resistance to the disease. After QTL mapping and physical representation of the particular chromosomal fragment spanning *Tir2* and 3, possible candidate genes were selected. Plausible candidate genes within the loci include TLR1, 5 and 6. The efficiency of clearance of the first wave of parasitemia in mice infected with *T. congolense* is positively correlated with long term survival. Rapid defense mechanisms are on the other hand provided through recognition of pathogen associated molecular patterns by receptors such as TLRs. Their expression appears to be essential for the induction of interleukins such as IL-10 and TNF- α . They act singly or in synergy in regulating macrophage activation status. Susceptible and resistant mouse strains portray differential expression of IL-10 and TNF- α over time, which might be dependent on the expression of Tolls. In order to investigate the mode of expression of the TLR, eleven groups of mice were selected and bred to maturity. Two groups of mice were sacrificed and their spleen and liver tissues collected. The other nine groups were subsequently challenged with *T. congolense* strain IL1180 and liver and spleen tissues collected at day 3, 4, 7, 10, 13 and 17 post challenge. After RNA extraction and reverse transcription, quantitative and semi quantitative PCR methods were applied to determine TLR and β -actin mRNA expression patterns. Results were presented as a ratio of the target TLR normalized to β -actin as a house keeping gene.

TLR1, 5 and 6 were readily detectable in cDNAs prepared the tissues from the resistant and susceptible mouse strains. Analysis of variance of the mean ratios of the TLR target normalized to the house keeping gene revealed that the genes are regulated in a statistically significant fashion. The overall TLR1 expression could significantly distinguish pre- and post-trypanosome infection status in the liver and spleen tissues. Interestingly, the data also identified two distinct groups among the liver samples, which were either expressing or not expressing TLR1 gene before and after infection which was supported by the higher expression thereafter as evident from real time PCR results. Similar trends were observed with TLR6 gene over time since the levels of TLR6 increased at the onset of the infection in all the strains but were much higher in the susceptible A/J strain than Balb/c and C57BL/6J mouse strains. TLR5 gene was clearly expressed in the three mouse strains with C57BL/6J showing weak expression through out the experimental period. The susceptible A/J and Balb/c showed increased mRNA expression of TLR5. However higher levels were observed in Balb/c as opposed to A/J. Susceptible and resistant mice infected with *T. congolense* therefore portray diverse expression patterns of the TLR genes. Up regulation of different TLR seems to coincide with up regulation of IL-10 and TNF- α in susceptible and resistant strains respectively. Response to the *T. congolense* infection therefore induces a response characterized by changes in TLR 1, 5 and 6 expression in liver and spleen tissues which in turn may influence different cytokine patterns in mice. Determination of TLR levels in resistant and susceptible livestock can therefore be exploited in the development of novel strategies for the control of trypanosomiasis in man and his livestock.

Complex Traits

Oral Presentation

Tuesday November 8

2.30pm – 2.45pm

O-34

FOUR –AT-A-TIME APPROACH TO THE EXTENDED RIX DIALLEL CROSS AND F2 GENERATION OF THEIR PROGENITOR STRAINS: NEW TOOL FOR GENETIC DISSECTION OF QUANTITATIVE COMPLEX TRAITSA V Osadchuk¹, YuV Baburov¹, D C Airey², Lu Lu³, D W Threadgill⁴, R W Williams³¹ Institute of Cytology and Genetics, Novosibirsk, Russia, ² Vanderbilt University, Nashville, TN, United States,³ University of Tennessee, Memphis, TN, United States, ⁴ University of North Carolina, Chapel Hill, United States

We have developed an effective statistical approach to study complex traits that are modulated by multilocus systems with epistatic interactions. The approach was tested in the context of a large but incomplete RIX diallel generated from the CXB strain set and a complementary BALB/cByJ x C57BL/6ByJ F2 population. The phenotype was cerebellar weight (CW). The analytical suite consists of six statistical tests. 1. Multiple regression analysis was used to evaluate segregation models characterized by minimal number of loci that can account for among-line variation relative to within-line environmental noise. A beam search procedure with a priority queue size of up to 1.5 million different 4-locus genotypes was used to extract adequate solutions. 2. We used a multilocus search procedure to evaluate linkage between these models and sets of marker loci. Based on the segregation model, genotypic CW values for all 81 RIX lines were estimated. 3. We then used a G test to compare expected and observed distributions of F2 phenotypes. 4. The similarity between observed and predicted CW values for non-recombinant intervals in the F2 were tested. 5. 4-marker multiple regression was tested for all variants of flanking markers in the F2. 6. The similarity between all 81 genotypic values produced by segregation models and 4-marker multiple regression in the F2 was tested. Twenty-six 4-locus solutions were not rejected by the above tests. The major factor preventing convergence on a single solution is the severe non-syntenic association and the partial diallel. However, a simulation study revealed that it is possible to achieve a single solution by increasing the number of RI strains used to generate the RIX and by producing a complete diallel cross.

(The work was partly supported by the grant of BRP of the PRAS)

Complex Traits

Oral Presentation

Tuesday November 8

2.45pm – 3.00pm

O-35

A SYSTEMATIC GENETIC DISSECTION OF DIET-INDUCED METABOLIC SYNDROME USING THE B6-CHR^A CHROMOSOME SUBSTITUTION STRAIN PANEL OF MICE.

D S Sinasac, M E Slaughter, S K Iyengar, J H Nadeau
Case Western Reserve University, Cleveland, Ohio, United States

The metabolic syndrome (MetS) is a cluster of obesity-associated risk factors for cardiovascular disease, diabetes and stroke. Despite the established consequences of diet and lifestyle on MetS, the underlying genetic susceptibilities in humans remain elusive. Male C57BL/6J mice fed a high fat/high sucrose (HF/HS) diet represent a diet-induced model of MetS, while other inbred strains like A/J appear resistant. To systematically dissect the genetics of diet-induced MetS, a screen examining traits related to obesity, insulin resistance, dyslipidemia and fatty liver in the B6-Chr^A chromosome substitution strain (CSS) panel is currently underway. When completed, the screen will consist of 30 males per strain (A/J, (B6XA/J)F1, C57BL/6J and 22 CSSs) maintained for 16 weeks on the HF/HS diet, fasted overnight, bled and sacrificed for tissue. Preliminary observations have revealed that, in addition to strain differences in weight gained on the HF/HS diet, strong correlations exist between many of the MetS traits across strains. To determine if the relationships between MetS traits within a strain might differ by strain, principal components analyses were performed on data for 13 CSSs and C57BL/6J, followed by ANOVA to compare component loadings for each principle component. The analyses indicated that 10 of the CSSs have similar relationships compared to C57BL/6J, while three of the CSSs comprise 2 groups whose MetS trait relationships are both different from C57BL/6J and each other. These results suggest that genetic susceptibility to MetS may act through either a propensity for weight gain or through modulation of the physiological relationships between MetS traits.

Complex Traits

Oral Presentation

Tuesday November 8

3.00pm – 3.15pm

O-36

MATERNAL GENOTYPE AFFECTS ADULT OFFSPRING LIPID, OBESITY, AND DIABETES PHENOTYPES IN LGXSM RECOMBINANT INBRED STRAINSJ P Jarvis¹, J Kenney-Hunt¹, T H Ehrich¹, L S Pletscher¹, C F Semenkovich², J M Cheverud¹¹ Washington University School of Medicine, Department of Anatomy and Neurobiology, St. Louis, MO, United States,² Washington University School of Medicine, Department of Medicine, St. Louis, MO, United States

Maternal effects on offspring phenotypes occur because mothers in many species provide an environment for their developing young. While these factors are correctly “environmental” with respect to the offspring genome, their variance may have both a genetic and an environmental basis in the maternal generation. Here, reciprocal crosses between C57BL/6J and 10 LGXSM recombinant inbred (RI) strains were performed and litters divided at weaning into high and low fat dietary treatments. Differences between reciprocal litters were used to measure genetic maternal effects on offspring phenotypes. Nearly all traits, including weekly body weights and adult blood serum traits show effects indicative of genetic variation in maternal effects across RI strains allowing the quantitative trait loci (QTL) involved to be mapped. Though much of the literature on maternal effects relates to early life traits, we detect strong and significant maternal effects on traits measured at adulthood (as much as 10% of the trait variance at 17 or more weeks post-weaning). We also found an interaction affecting adult phenotype between the effects of maternal care between RI strain mothers and C57BL/6J mothers and a later environmental factor (dietary fat intake) for some age-specific weights.

Complex Traits

Oral Presentation

Tuesday November 8

3.15pm – 3.30pm

O-37

INSIG2 : A CANDIDATE GENE FOR CHOLESTEROL REGULATION

A Cervino², G Zastrow-Hayes², J Zhu¹, M Pletcher², N Tsinoremas², E Schadt¹

¹ Rosetta/Merck, Seattle, WA, United States, ² Scripps Florida, Jupiter, FL, United States

We followed up on a previously identified linkage region from a B6 and C3H/- F2 cross by testing for in silico association using our high density SNP map. In this study we genotyped 10,657 SNPs from 62 inbred strains. By integrating quantitative trait locus (QTL) mapping methods and in silico analysis, we identified *insig2* as a candidate gene for total plasma cholesterol levels.

Furthermore, using expression analysis, we reconstructed a genetic pathway that included known and novel genes involved in cholesterol synthesis. To validate the network in humans, we performed siRNA of *insig2* in HepG2 cell lines. Affymetrix genechips were run on cells that showed a 61% and a 59% knockdown according to the real-time PCR data. The knock down results confirmed the functional role of *insig2* in the cholesterol pathway in both human and mouse.

Complex Traits

Oral Presentation

Tuesday November 8

4.00pm – 4.15pm

O-38

STUDYING DOSAGE EFFECTS FOR HUMAN CHROMOSOME 21 HOMOLOGOUS GENES USING CHROMOSOMAL ENGINEERING IN THE MOUSEV Besson¹, A Duchon¹, V Brault¹, L Magnol¹, J-C Bizot², L Dauphinot³, M-C Potier³, Y Herault¹¹ CNRS Institut de Transgenose, Orleans, France, ² Key-Obs, Orleans, France, ³ CNRS-ESPCI, Paris, France

Human chromosome 21 (HSA21) is associated with two syndromes that depend upon gene-dosage balance: the Trisomy 21 (or Down syndrome) and the Monosomy 21. Both syndromes lead to different set of features affecting various organs like the skeleton, the heart, the gastrointestinal tract and the nervous system. In the mice, the homologous regions to HSA21 are found on three distinct chromosomes: 10 (MMU10), 16 (MMU16) and 17 (MMU17). The most commonly used model, Ts65Dn, corresponds to a subpart of MMU16 and displays some features of the Down syndrome, but does not resume the complete panel of alterations found in human patient. So we develop mouse models for the centromeric and telomeric HSA21 regions in order to establish a genotype-phenotype relationship. The corresponding models display a deletion (or a tandem duplication) of the homologous regions to HSA21 leading to the corresponding Monosomy (or Trisomy) in the mouse. In this meeting, we will present the preliminary analysis of mutant models carrying deletion or duplication of the regions of interest trying to show how these genetic configurations are helpful to locate genes with dosage effects homologous to the human chromosome 21 genes.

O-39

GENETIC AND PHYSIOLOGICAL ANALYSIS OF INTESTINAL LENGTHENING IN MOUSE STRAINS

S Bellier¹, G Aubin-Houzelstein¹, N Da Silva¹, JM Vanderwinden², X Montagutelli³, J J Panthier³

¹ Ecole Nationale Vétérinaire d'Alfort INRA, Maisons-Alfort, France, ² Université Libre de Bruxelles, Bruxelles, Belgium, ³ Institut Pasteur, Paris, France

How does an organ grow to a specific size ? Is the regulation of the size specific to each organ ? George Cuvier (1835) stated, in what is commonly referred to as the *law of coexistence*, that “ a living organism is a unique and self-contained whole. All of its parts are mutually related and cooperate for the same purpose. Although no part can change unless the others also change, each part considered separately suffices to indicate the others ”. PRM/Alf mice stand in contradiction to this law. They exhibit an obvious and selective lengthening of the intestine ; their intestine is 74.8 ± 5.3 cm long compared with 51.0 ± 3.0 cm in other inbred mice, such as DBA2/J. This unusual phenotype is acquired postnatally, before weaning. We investigated the functional consequences of such a lengthening and found that physiological mechanisms compensate for this anatomical variation. Genetic crosses showed that intestinal length is inherited in a polygenic way. More interestingly, cross-adoption of progeny to surrogate mothers revealed that the dam's genotype acted synergistically with the offspring's genotype to confer the longest intestine. In other words, maternal effects strongly contribute to intestinal lengthening in PRM/Alf mice. Two mutually non-exclusive mechanisms could well account for the maternal effects. First, the intestine of PRM/Alf mice could contain a specific society of indigenous gut microorganisms (microbiota). Germ-free PRM/Alf mice are being produced to test this hypothesis. Second, the milk of PRM/Alf females could contain intestinotrophic factors. Characterization of PRM/Alf milk proteins is carried out using proteomic techniques.

Complex Traits

Oral Presentation

Tuesday November 8

4.30pm – 4.45pm

O-40

DISSECTING THE GENETIC AND EPIGENETIC EFFECTS ON TUMOR SUSCEPTIBILITY IN A MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 1K M Reilly¹, R G Tuskan¹, K W Broman², S Tsang³, D J Munroe³¹ NCI-Frederick, Frederick, MD, United States, ² Johns Hopkins School of Public Health, Baltimore, MD, United States, ³ SAIC-Frederick, Frederick, MD, United States

Neurofibromatosis type 1 (NF1) is a familial disease of the nervous system with predisposition to cancer. It affects 1 in 3500 people, regardless of race or ethnicity. The clinical heterogeneity of NF1 and the potential role of modifier genes in determining the severity of the disease present serious challenges to patients and clinicians. We are using a mouse model of NF1 (NPCis mice) to understand the role of modifier genes in the malignancies associated with NF1. NPCis mice develop many of the malignancies associated with NF1, in particular glial tumors of the central and peripheral nervous system (secondary glioblastoma and peripheral nerve sheath tumors (PNST), respectively). We have found imprinting effects linked to chromosome 11 and strain-specific modifiers that affect the incidence of these tumors. The effect of imprinting on chromosome 11 has opposite parental effects on glioblastomas and peripheral nerve sheath tumors. We have analyzed the genetic interaction between an imprinted locus on mouse chromosome 11 and the modifier loci for nerve sheath tumor resistance, *nstr1* on mouse chromosome 19 and *nstr2* on mouse chromosome 15. The imprinted locus interacts epistatically with *nstr1* and *nstr2* to affect resistance to PNSTs. In the case of NPCis mice inheriting the mutant chromosome 11 from their father, loci on chromosome 19 in the A strain background act to increase resistance to PNSTs. In the case of NPCis mice inheriting the mutant chromosome 11 from their mother, a locus on chromosome 15 in the A strain background further increases the resistance to PNST. The relevance of these results to human NF1 patients is supported by the overlap of these loci with regions altered in PNSTs. Consistent with these loci being low-penetrance modifiers for PNST susceptibility, these genomic regions are altered in a relatively low number of mouse and human tumors. However, the observation that these regions are translocated in multiple human tumors suggests that these rearrangements are not random. Identification of modifiers of NF1 in mouse models will allow these modifiers to be tested directly in human association studies, specifically in cases where tumors can be tested for the change in expression of candidate imprinted genes. These data demonstrate that modifier genes affect tumorigenesis under very specific conditions. The understanding of these conditions will allow for more accurate risk assessment and genetic counseling for individuals at high-risk for cancer, and better targeting of cancer therapies based on the genetic and epigenetic alterations occurring within an individual tumor.

O-41

FUNCTIONAL GENOMICS OF MOUSE ACTIVITY AND ANXIETY – A MULTI-DIMENSIONAL SURVEY

L Liu, C Fernandes, F V Rijdsdijk, J L Paya-Cano, M J Parsons, L C Schalkwyk
Institute of Psychiatry, King's College London, London, United Kingdom

Behavioural traits, such as anxiety, baseline and exploratory activity, are quantitative and complex, being determined by multiple and interactive genetic and environmental components. There is also a strong interaction between the traits in that exploratory activity plays a pivotal role in anxiety tests, yet it is unclear to what extent these two traits overlap in terms of their genetic and environmental sources of variation. We applied a large behavioural test battery consisting of baseline activity, exploratory activity and anxiety paradigms, to four different populations of mice: C57BL/6J mice (n=100, in 10 groups different environmental manipulations), a panel of 8 standard inbred strains (n=10/strain), 24 BxD RI lines (n=279) and >600 Boulder HS mice consisting of full sibs and half sibs. We will report our research on the following four dimensions with emphasis on the dissection of activity and anxiety: 1) Quantitative assessment of environmental variation and heritability of the behaviour measures. In addition to the inbred mice, we also used the HS population to assess the structural relationship between activity and anxiety measures in terms of their unique and shared environmental as well as additive genetic effects; 2) We made extensive use of the WebQTL tool on data collected from the BXD panel, this includes *in silico* QTL mapping, genetic correlation analyses with published phenotypic traits and brain gene expression from WebQTL; 3) Association study on GABA A receptor genes in the HS population; 4) hippocampus expression profiling using Affymetrix gene chips on HS individual mouse selected on phenotypic variation.