

P100

DEVELOPMENT OF DATABASE FOR EXPERIMENTAL PARAMETERS OF REPRODUCTIVE TECHNOLOGIES

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Reproductive technology is widely used for production and preservation of experimental mice. There are substantial varieties of the procedures used for the reproduction of mice in different laboratories. The variations are due to difference in experimental or rearing conditions such as environments of animal housing (temperature, moisture and noise for breeding room), materials (mouse strains, equipments and reagents) and methodologies (*in vitro* fertilization, superovulation, sperm and oocyte collection, insemination and culture medium, etc). It has been well recognized that direct comparison of the detailed procedures used among different institutions and laboratories is needed to identify the cause of difference in efficiency of reproduction, and to improve the procedure of each laboratory. We developed a new format to describe every procedure for mouse reproduction technologies, which provides framework of procedure descriptions. This format is referred to as "Standardized Description of Operating Procedures (SDOP)" Through efficient international sharing of protocol information, it enables us to easily identify differences in parameters of the reproductive technologies used in different institutions and laboratories. It may be useful even for management of different versions of protocols used within individual laboratory. SDOP would be also useful to manage the reproduction procedures operated in mouse resource centers.

P101

MUTANT MODELS FOR ESOPHAGEAL CARCINOGENESIS IN RIKEN MOUSE ENU-MUTAGENESIS PROJECT

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In order to establish mouse mutant lines modeling human carcinogenesis and other common diseases, we have performed dominant screening in RIKEN ENU mutagenesis project. After the end of early-onset phenotype screening procedures in 13 weeks of age G1 mice, late-onset phenotype screenings were commenced and to be continued until 78 weeks of age. During this period, anatomical, histological and hematological analysis were performed as the screening procedures to discover neoplastic lesions in every organs and hematic system. We have screened 5,000 G1 mice from mutagenized C57BL/6J male mice crossed to DBA/2J females in the late-onset stage. By this screening procedure, 4 mutant candidates of esophageal cancer phenotype were identified. For inheritance testing and mapping of these candidates, we produced G2 generations by backcrossing G1 mutant candidates to DBA/2J animals to show clear inheritances. Mapping for the causative gene with introduced mutation were performed using SNPs markers and the esophageal cancer phenotype of 4 mutant lines showed linkages on chromosomes 5. The results of further morphological and genetic analysis will be reported.

P102**ANALYZING MULTIPLE MOUSE LINES POSSESSING POINT MUTATION ON BETA-CATENIN (CTNNB1) GENE**

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ENU-based gene driven mutagenesis is yet another reverse genetics by identifying point mutation of specific genes from about eight thousands of ENU-mutagenized mouse genome archive. Once identifying mutation, we can recover mutant line from frozen sperm. As compared with knockout, this is cost-effective to obtain multiple alleles at the same time; while we do not know how much percent of given allelic mice display "phenotype." Beta-catenin (ctnnb1) is bifunctional protein; Wnt-signaling compartment and structural molecule. It interacts many kinds of proteins with well-conserved domains spreading almost entire molecule. Thus, analyzing a series of alleles of this gene is typical case to know the phenotype spectrum, as well as searching new biological function. About screening with primer sets covering about half of this gene resulted in identifying twelve mutations; 3 intronic, 6 missense, 1 nonsense, and 2 synonymous. Six missense and one nonsense mutants were subjected to the evaluations of their phenotype in vitro and in vivo. We are evaluating in vitro by introducing a series of mutant cDNA to cultured cell line. Four mutant mouse lines have recovered so far and been evaluated in vivo. First we tested embryonic lethality and checked appearance of offspring, according to the reports that null mutant is embryonic lethal and that part of conditional alleles show abnormal appearance, such as coat, eye, and so on. We found that part of nonsense heterozygote had kinked tail and homozygote died around embryonic day 9.5, and that one missense allele may show embryonic lethality.

P103**RIKEN BRC TO ESTABLISH MOUSE RESOURCES OF THE HIGHEST GLOBAL STANDARDS**

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The mice are one of the most important model organisms in life sciences. Large-scale strategic knockout mouse projects have been launched by the USA, Canada and EU countries to target every gene for establishment of research resources of the nations. RIKEN BRC has been designated as a central core facility for mouse resources in Japan by the National BioResource Project since 2002. With support of scientific community, RIKEN BRC has collected over 2,800 strains including unique genetically-engineered strains, ENU mutants, inbred and wild-derived strains developed mainly in Japan. RIKEN BRC has established a reliable system for collection, preservation, quality control and distribution of mouse resources. RIKEN BRC is one of the founding members of the Federation of International Mouse Resources (FIMRe), and has participated in the International Mouse Strain Resource (IMSR), a global one-stop shop of mouse strains worldwide. RIKEN BRC will continue its strategic plan to collect novel models for human diseases and gene functions. All mice in our facility are cleaned-up to specific pathogen-free state, strictly monitored for their health, and accurately tested on genetic modification and background of the strains. Phenotypic information of the strains is collected to enrich their value. Thus RIKEN BRC plans to establish mouse resources of the highest global standards in quality and quantity by 2010. RIKEN BRC protects by using MTA the intellectual property rights of the developer of the strains and promotes distribution of the mice. Training courses are also provided to disseminate advanced technologies for best use of mouse resources.

P104**EVALUATION OF ENU-INDUCED MUTATION SCREENING SYSTEM BY NON-FLUORESCENT TILLING METHOD**

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In the RIKEN ENU mouse mutagenesis project, we have produced the frozen sperm archive from over 10,000 G1 male offspring. So far, about 8,000 G1 genomic DNAs have been extracted and archived. Thus the sequence-based mutagenesis for particular genes of the interest has become available. This system can be used as a reverse genetics tool to analyze the gene function through various mutant mice. To detect the mutation in target genes from the archive, we have adopted the TGCE method that can efficiently distinguish heteroduplex from homoduplex DNA fragments. We are now able to detect more than 100 point mutations per year. To enhance the mutation detection efficiency and to accelerate the functional gene analysis in the entire mouse genome, we have been trying to adopt the non-fluorescent TILLING (nf-TILLING) method. The fluorescent TILLING (f-TILLING) method has a high sensitivity and efficiency for the mutation detection. The f-TILLING also has some disadvantage that it has to use the expensive fluorescent primers and tends to show some background noises presumably by unexpected exonuclease activity of *Cel* I. On the other hand, the nf-TILLING exhibits lower background noises than the f-TILLING, because any residual primers and their nonspecific single-stranded byproducts are quiescent. We have prepared many positive control samples, some of which were the discovered mutations from our archive. We also constructed plasmid clones as positive controls by PCR-based replication errors. We report the performance of the nf-TILLING for the mutation discovery with various conditions including the sensitivity in the pooled DNA samples.

P105**ESTABLISHMENT OF EMBRYONIC STEM CELL LINES DERIVED FROM MSM/MS STRAIN ORIGINATED FROM MUS MUSCULUS MOLOSSINUS.**

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The MSM/Ms strain was established from Japanese wild mice, *Mus musculus molossinus*, collected in 1978 in Mishima, Japan. Comparison of the MSM/Ms sequences with the C57BL/6J revealed that the overall nucleotide substitution rate as high as 0.96%, which is quite big if compared with the genomic difference between humans and chimpanzees (1.23%). MSM/Ms has unique characteristics not observed in the commonly used laboratory strains, for example, extremely low incidence of tumor development, characteristic behavioral phenotypes and resistance to high-fat diet-induced diabetes. Therefore, functional genome analyses in MSM/Ms should provide a means to identifying novel phenotypes and gene functions. We report here the derivation of germline-competent ES cell lines from MSM/Ms blastocysts, allowing the *Mus musculus molossinus* genome to be genetically manipulated. Using Knockout Serum Replacement (KSR) and Glasgow minimal essential medium (GMEM), we found that we can readily establish ES cell lines from blastocysts. Three lines (Mol/MSM-1, 2 and 3) were established and tested for chimera generation by aggregation with ICR mofura. In all three lines, chimeric mice were born, and coat-color chimerism varied from 5-100%. High percentage chimeras were used for in vitro fertilization with ICR oocytes and confirmed germline transmission. We also injected Mol/MSM-1 ES cells into blastocysts of ICR or C57BL/6 X BDF1, and found that injection into C57BL/6 X BDF1 gave higher production rate of chimeric mice. These Mol/MSM ES lines provide alternative tool for mutagenesis and analyses of phenotypes.

P106**ENU-BASED GENE-DRIVEN MUTAGENESIS IN THE MOUSE: MUTATIONS IN CODING/NONCODING AND TRANSCRIBED/NONTRANSCRIBED SEQUENCES**

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It is now possible to generate mutant mice having ENU-induced point mutations in a specific target gene by gene-driven mutagenesis. We have prepared frozen sperms and genomic DNA from about 8,000 G1 male mice for this purpose. Over 400 mutations have been found mainly by the Temperature Gradient Capillary Electrophoresis (TGCE) method and many mutant mice have been recovered from the frozen sperm archive. We already have reported that this method is very useful for the functional analysis of coding genes (e.g., Clapcote et al. *Neuron* 2007, 54(3):387-402) and also noncoding sequences (e.g., Masuya et al. *Genomics* 2007, 89(2):207-214). We will present the overview of this gene-driven mutagenesis. Last year we presented the mutation rate in the Long Conserved Noncoding Sequences (LCNS) are equivalent to that of coding sequences. Together with further analyses LCNS are shown to be evolutionary preserved because of the selection pressure, indicating some biological functions of LCNS. On the other hand, we have found that mutation rate of A/T site in transcribed strands is significantly lower than that of nontranscribed strands. This is an evidence for the transcription coupled DNA repair (TCR) of ENU-induced lesions in the mammalian genome. These studies have become plausible due to the direct identification of ENU-induced mutations in the mouse genome.

P107**HAIR MORPHOLOGICAL MUTANTS GENERATED IN THE RIKEN MUTAGENESIS PROJECT**

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In the RIKEN mouse ENU-mutagenesis program, we have generated twelve hair morphological mutants that exhibit curled, waved hair coat or vibrissae. Gene mapping assigned these mutants to a single chromosomal region, respectively. We explored candidate genes of the mutants by searching for the knowledge database "PosMed" (<http://omicspace.riken.jp/PosMed/>), and extracted strong candidates of almost all the mutants. Direct sequencing of them revealed genetic alterations in genomes of the mutant mice. Thus far, we successfully identified point mutations in five genes of the nine mutants, such as keratin25 (*Krt25*), keratin71 (*Krt71*), epidermal growth factor receptor (*Egfr*), hairless (*Hr*) and serum/glucocorticoid regulated kinase 3 (*Sgk3*) genes. These mutations in the candidate genes likely obstruct hair and hair follicle formation. In brief, the mutations of *Krt25* and *Krt71* cause unstable heterodimerization of the Type I and II keratin proteins in the inner root sheath (IRS) of hair follicle. The mutation of *Egfr* causes signal transmission disorder in cells in the outer root sheath (ORS) of hair follicle. The mutations of *Hr* and *Sgk3* cause dysregulation of the *Wnt* signaling in the hair cycle. Thus, it is most likely that the identified mutations are responsible for the phenotypes of the mutants.

S1-3/P108

IDENTIFICATION OF A NOVEL LOSS-OF-FUNCTION MISSENSE MUTATION IN THE RANKL GENE USING ENU MUTAGENESIS

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Biomedical Sciences Research Center Alexander Fleming

Bone-related diseases such as osteoporosis, rheumatoid arthritis or cancer metastasis are characterized by increased osteoclast activity. RANKL is an essential, central regulator of osteoclast development and function and its expression is upregulated in a broad variety of bone-related diseases. Blocking RANKL appears to be the most efficient and relevant approach for the treatment of diseases associated with bone loss.

We have recently isolated an ENU-induced mouse mutant of osteopetrosis, which is characterized by loss of tooth eruption, abnormally increased bone density, and complete absence of osteoclasts. Genetic analysis using genome-wide polymorphic markers, SSLPs and SNPs, have led to the localization of the causal mutation in distal chromosome 14 at a genomic interval including the RANKL gene. Sequencing analysis of the RANKL coding region revealed a missense mutation, which caused a single aminoacid substitution in a highly conserved region of the extracellular domain. Functional characterization of the mutated inactive protein provides initial evidence that the mutation affects RANKL binding to its cellular receptor RANK or the decoy receptor OPG. This knowledge provides new possibilities for designing drugs to inhibit RANKL function.

P109

IDENTIFICATION OF GENETIC MODIFIERS USING RANDOM MUTAGENESIS IN MODELED RHEUMATOID ARTHRITIS AND INFLAMMATORY BOWEL DISEASE

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Genome-wide, sensitized ENU mutagenesis screens on mouse models of human diseases offer unique opportunities to isolate progeny with disease attenuation and subsequently discover novel targets directly associated with prevention or therapy of the relevant diseases. We have thus initiated a program of sensitized ENU mutagenesis screen applied on our established TNF^{ΔARE} model of Rheumatoid Arthritis and Crohn's-like Inflammatory Bowel Disease (IBD), to identify novel genes that modify the development of these diseases. By screening 6318 G3 mutagenized TNF^{ΔARE} offspring we have identified three families that demonstrated a significantly delayed onset and progression of arthritis and six families with dramatic attenuation of IBD. So far, a wide genome linkage analysis approach using both SNPs and SSLPs, identified two chromosomal loci associated with arthritis attenuation and one chromosomal locus associated with attenuation of IBD. Further narrowing of the candidate regions of interest coupled with direct sequencing of candidate genes should identify the gene(s) responsible for the disease neutralizing effects in these models.

P110**ESTABLISHMENT OF ENU-INDUCED DOMINANTLY INHERITED RETINAL DEGENERATION IN MICE**

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Retinitis pigmentosa (RP) is one of the genetically heterogeneous disorders characterized by progressive night blindness and loss of peripheral vision resulting from degeneration of photoreceptors. Our aim is to establish animal models for RP and identify the responsible genes in RIKEN mouse ENU-mutagenesis program. Funduscopic examination was carried out on about 2,500 individuals for dominant screening. As a result, 36 individuals showed various abnormal ocular phenotypes. Within four dominantly inherited mutants having retinal abnormalities, two mutants exhibited progressive retinal degeneration (RD) which is equivalent to human RP. Between the two mutants, the degree of retinal degeneration was very different. We have analyzed the histopathology of retina, electroretinograph, and the function of causable genes in these mutants.

P111**MICE OF ALL TYPES: INTEGRATING PHENOTYPIC DATA FOR SCREENS, MODELS, AND COMPLEX GENOTYPES**

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The Jackson Laboratory

Myriad projects to phenotype mice of defined genetic backgrounds or carrying single or complex combinations of spontaneous, induced, and genetically engineered mutations are being done at centers performing large-scale screens and by researchers doing detailed morphological, molecular and functional analyses. To maximally utilize this work, organized primary typing data and integrated outcome data are required. The Mouse Genome Informatics Database (MGI, www.informatics.jax.org) integrates descriptions of phenotypes and metadata about mutations and genotypes; and creates a powerful environment for data querying and mining in concert with other available genetic, genomic, and biological data. Use of structured vocabularies for anatomy, gene function (GO), phenotypes (Mammalian Phenotype Ontology), and diseases (OMIM); and adherence to nomenclature standards enables rich annotation. In July 2007, MGI included >92,000 phenotype annotations involving >18,000 unique genotypes. MGI phenotype data acquisition will accelerate as null and conditional null mutants from international knockout mouse projects are systematically phenotyped. Primary data being capturing from screens of individual mice from inbred and recombinant strains and mutagenesis projects currently are widely dispersed and only some are accessible through public databases, including MPD (Mouse Phenome Database, www.jax.org/phenome) and Europhenome (www.europhenome.eu/) for strain characteristics, MGI for public Deltagen and Lexicon knockouts (www.informatics.jax.org/external/ko/), and RIKEN (www.gsc.riken.go.jp/Mouse/main.htm) and others for ENU mutagenesis data. These growing data challenge the community to organize data centers, formulate data standards, and establish common data formats. MGI's phenotypic outcome data and existing primary data resources will be described, as well as challenges of future phenotype data coordination. Supported by NIH grant HG000330

P112**WHERE IN THE WORLD? USING IMSR TO FIND MOUSE RESOURCES**

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The International Mouse Strain Resource (IMSR) facilitates access to mouse resources and models of human disease used for basic and translational research. The unique and pivotal role of IMSR is in unifying information about mouse resource holdings worldwide, including inbred, mutant, and genetically engineered mice maintained as breeding stock; cryopreserved embryos, ovaries and sperm; and ES cell lines. As of July 2007, IMSR tracks over 10,000 strains and stocks from 17 repository consortia representing 27 individual repositories in the U.S., Canada, Europe, Japan, and Australia. Additionally, nearly 28,000 mutant ES cell lines from BayGenomics, Sanger Institute, and the Nagy and Soriano laboratories are included. All repositories belonging to FIMRe (Federation of International Mouse Resources, www.fimre.org) list their holdings in IMSR. At the IMSR website (www.imsr.org), users can search for mouse resources, locate holding repositories, link to details about strains or cell lines, such as maintenance and availability, contact a repository for ordering, and follow links to associated phenotype and disease model descriptions. Users of MGI (www.informatics.jax.org) also will find links directly to relevant IMSR listings from Phenotype Detail pages. IMSR will further expand with new resource listings from the International Knockout Mouse Projects (KOMP / EUCOMM / NORCOMM) and International Gene Trap Consortium; and five additional new repositories joining the IMSR database. In this presentation we will illustrate IMSR functionality and describe how planned enhancements will continue to put new resources at researchers' fingertips.

P113**EXAMINATION OF THE SPATIAL AND TEMPORAL EXPRESSION OF SICKLE TAIL (SKT) GENE IN NOTOCHORDAL CELLS.**

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The notochord functions as a signaling center during embryogenesis. The notochord has major roles in vertebral column formation: indirectly by inducing sclerotome cell differentiation, and directly by forming the nucleus pulposus (NP) of intervertebral discs. We previously reported that the murine Sickle tail (Skt) is specifically expressed in the embryonic notochord and adult NP cells of the IVD. The Skt gene is identified by the analyses of SktGt mice. The SktGt is a transgenic mouse line established by the gene-trap method. The β -galactosidase, a reporter molecule, derived from SktGt is expressed dependent upon the Skt promoter and is detected in the notochord during embryogenesis and in the NP of mice in entire life. Until now, any gene that keeps expressing specifically in the notochord lineage cells in entire life is not reported. Our results indicate that the Skt gene is expressed specifically in notochordal cell lineage and the Skt is a candidate for molecular marker to monitor distribution of notochordal cells during development and in adult mice. In this study, we performed the detail examination of the spatial and temporal expression of Skt gene in embryos and adult mice, and investigated whether the Skt was a molecular marker of notochordal cell. The activity of β -galactosidase in the SktGt embryos and adult mice was detected clearly in the notochord lineage cells in entire life; i.e., embryonic notochordal cells and the remnant, NP cells. This result suggested strongly that the Skt was a molecular marker of notochordal cell lineage.

P114**GENE-DRIVEN SCREEN OF AN ENU ARCHIVE OF MOUSE DNA AND SPERM.**

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Gene driven screens of mutagenized mice represent a powerful tool in the identification of point mutations within the mouse genome.

ENU (N-ethyl-N-nitrosourea) introduces random mutations throughout the mouse genome at a relatively high efficiency. Male C57BL/6J mice were exposed to ENU and used to create a mouse mutant resource consisting of two parallel archives of frozen sperm and of DNA. Once a mutation is identified, then mutant mice can be rederived easily by *in vitro* fertilisation (IVF) and the resulting progeny used for establishing a mutant line and for phenotyping. A gene based screen of over 90 Mbp of DNA was carried out using various platforms: Denaturing high performance liquid chromatography (WAVE), Temperature gradient capillary electrophoresis (SpectruMedix) and Hi-resolution melting detection (Light Scanner). We have identified 84 mutations of which 53 were potentially functional. The mutation rate is 1 in 0.93 Mbp of screened coding DNA. For 40 alleles mutant mice have been recovered from the sperm archive by IVF and the presence of the predicted mutation confirmed in the resulting offspring. As ENU induces random point mutations it can be expected that the full range of functional mutations in any gene might be uncovered including amorphs (null), hypomorphs, hypermorphs and neomorphs.

The gene-driven screen, based on the use of an archive of mouse ENU DNA and sperm, is a powerful adjunct to conventional mutagenesis strategies. It has the advantage of rapidly recovering a variety of alleles, with potentially different phenotypic outcomes, that facilitate the investigation of gene function.

The archive consists of over 6,700 samples and continues to grow, with a target of 10,000 in the near future; it is available to academic collaborators as a community resource and requests for DNA should be made to: gems@har.mrc.ac.uk

<http://www.mgu.har.mrc.ac.uk/facilities/dnaarchive/>

P115**AN E3 UBIQUITIN LIGASE, RNF41, IS ASSOCIATED WITH ANXIETY-LIKE BEHAVIOR, MAJOR DEPRESSION, AND BETA-CARBOLINE-INDUCED SEIZURE**

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The molecular etiology of individual differences in complex behavior traits and susceptibility to psychiatric illness remains incomplete. Using an unbiased genetic approach in a mouse model, Quantitative Trait Loci (QTL) influencing anxiety-like behaviors and beta-carboline-induced seizure vulnerability have been mapped to the distal portion of mouse chromosome 10 and an interval specific congenic strain (ISCS; A.B6^{chr10}; 66 cM to telomere) was developed. This A.B6^{chr10} strain facilitated defining the behavioral influences of this region as well as gene expression profiling to identify candidate gene(s) underlying this QTL. By microarray studies, an unsuspected E3 Ubiquitin Ligase, Ring Finger 41 (*Rnf41/Neuregulin Receptor Degrading Protein1; Nrdp1*) was differentially expressed in the region of interest, comparing the hippocampi of A/J vs A.B6^{chr10} mice as well as A/J vs B6 mice. By RT-PCR, *Rnf41* expression levels were significantly increased 1.5 and 1.3-fold in the hippocampi of C57BL/6J and A.B6^{chr10} mice compared to A/J mice, respectively. In addition, protein levels of Rnf41 were increased in hippocampi of B6 mice compared to A/J mice across postnatal development with a 5.5-fold difference at P56. Re-analyzing a microarray database of human post-mortem prefrontal cortex (Brodmann's Area 46/10), *RNF41* mRNA expression levels were reduced significantly in patients with major depression and bipolar disorder compared to unaffected controls. Overall, *Rnf41* is a pleiotropic candidate gene for anxiety-like behaviors, depression, and vulnerability to seizures. RNF41 and its binding partners provide novel etiological pathways for influencing behavior, highlighting a potential role for the ubiquitin proteasome system in psychiatric illness.

P116

PROTEOMIC ANALYSIS REVEALS MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATION-PRONE MICE LACKING MAHOGUNIN RING FINGER-1

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Oxidative stress, ubiquitination defects and mitochondrial dysfunction are common mechanistic themes underlying neurodegenerative disorders. Mahogunin Ring Finger-1 (*Mgrn1*) and *Attractin* (*Atrn*) null mutant mice develop age-dependent spongiform degeneration of the central nervous system. Based on phenotypic similarity, it has been suggested that MGRN1 and ATRN act in the same signaling pathway. *Mgrn1* encodes an E3 ubiquitin ligase, leading us to hypothesize that loss of MGRN1 causes neurodegeneration via aberrant accumulation of its substrates. Differences in brain protein expression between *Mgrn1* mutant and control mice were identified by proteomic analysis. Many proteins with mitochondrial functions showed reduced expression and functional assays confirmed reduced Complex I and Complex IV activity in mitochondria from *Mgrn1* mutants. Brain proteins from these mice also showed increased oxidative damage. Interestingly, these defects were apparent several months before histological signs of vacuolation. Compatible with the hypothesis that ATRN and MGRN1 act in the same pathway, mitochondrial dysfunction and increased oxidative stress were observed in *Atrn* mutant mice. Although the causal relationship between loss of ATRN or MGRN1 and mitochondrial dysfunction remains unclear, our results suggest that further study of ATRN and MGRN1 will provide insight into the molecular mechanisms that underlie other neurodegenerative diseases with similar defects.

P117

EFFECT OF FLUOXETINE ON THE BEHAVIOR OF MICE WITH ANXIOUS DEPRESSION UNDER PREVENTIVE AND THERAPEUTICAL ADMINISTRATION

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Novel technique of screening of psychotropic drugs under simulated clinical conditions (Kudryavtseva et al., 2007) could be used for evaluating the action of antidepressants and anxiolytics under chronic administration and for detecting protective and therapeutic effect of drugs. Effects of the well-known antidepressant fluoxetine, which is widely used in clinical treatment of depression, have been studied. Anxious depression was induced in the C57BL/6J male mice under chronic social stress evoked by repeated social defeats in daily intermale agonistic interactions. Under preventive administration, fluoxetine (25 mg/kg) was given orally to mice during two weeks starting from the 5th day of the social interactions, which continued during the whole period of treatment. Administration of fluoxetine on the background of continued social stress was shown to produce no positive effect on the state of males: the drug-treated mice manifested high level of anxiety, lowered communicative behavior and impaired motor and exploratory activities, indicating the lack of protective effect. Moreover, fluoxetine acted as a "pro-depressive" drug as demonstrated by Porsolt test. Under therapeutic administration, fluoxetine was given during 14 days to males with anxious depression formed after 20 days of social confrontations. Upon the cessation of agonistic interactions fluoxetine was found to produce an expressed antidepressant effect in males. In this case the drug also reduced the level of social withdrawal, i.e. enhanced the communicative behavior. It has been concluded that fluoxetine blocking the reuptake of serotonin is ineffective on the background of severe social stress, which is accompanied by the activation of the brain's serotonergic systems. However, under serotonergic system hypofunction developing in depressive individuals the drug may produce positive effect as early as two-three weeks after the beginning of treatment.

P118**CHROMOSOMAL ASSIGNMENT OF QTLs INFLUENCING MOUSE ANXIETY-RELATED BEHAVIOR IN THE MODIFIED HOLE BOARD USING CONSOMICS**Marijke C. Laarakker, Frauke Ohl, and Hein A. van Lith

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Male mice from a panel of chromosome substitution strains - in which a single full-length chromosome from the A/J inbred strain has been transferred onto the genetic background of the C57BL/6J inbred strain - and the parental strains were examined in the modified hole board test. With this behavioral test several parameters for anxiety-related behavior towards an unprotected area were measured/calculated. Both univariate and bivariate analyses were performed. The results agreed well with previous reports of QTLs for anxiety-related behavior using the A/J and C57BL/6J as parental strains. In addition, a novel QTL was found on the Y chromosome. While other researchers focus with respect to anxiety-related behavior on mouse chromosome 1, we have special interest for mouse chromosome 19. Combining the published data from multiple crosses between A/J and C57BL/6J it is most likely that the segment with coordinates 32,551,766 - 42,541,189 bp on mouse chromosome 19 contains the QTL(s) involved in anxiety-related behavior. By merging the QTL mapping data so far for this chromosome with those from a stock of genetically heterogeneous mice, we could narrow down the QTL interval on chromosome 19 to two segments (295 kb and 2635 kb). These two subregions together contain only a limited number (16) of genes.

P119**GENETIC DISSECTION OF MOUSE ANXIETY-RELATED BEHAVIOR**Marijke C. Laarakker, Frauke Ohl, and Hein A. van Lith

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The modified hole board (mHB), which is a complex behavioral test for rodents, allows us to assess for a variety of different motivational systems in parallel (i.e. exploration, locomotion, avoidance, arousal, memory, and risk assessment). This approach is essential for behavioral characterization since the motivational system of interest is strongly influenced by other behavioral systems. In previous experiments the C57BL/6J and A/J mouse inbred strains were behaviorally phenotyped in the mHB and showed differences in almost all motivational systems. To elucidate the genetic mechanisms underlying those behavioral differences, we performed further analyses with a commercially available set of mouse chromosome substitution (CS) strains. For this set C57BL/6J is the host strain and A/J is the donor strain. We identified one CS-strain (C57BL/6J-Chr19^A/NaJ) that differed in avoidance behavior (i.e. anxiety) from the C57BL/6J, but not in locomotion. Thus pleiotropic contribution of locomotion could be excluded. To identify which of the genomic regions that the CS-strain inherited from the A/J are responsible for this phenotype, an F₂-intercross between C57BL/6J and the CS-strain is currently produced. After quantitative trait loci analyses we hope to identify candidate genes and future work will be directed towards use of knockout strategies and micro-array analyses to assess the contribution of these candidate genes in relation to anxiety-related behavior.

P120**A GENETIC ANALYSIS OF ENU INDUCED MOUSE MUTANT THAT SHOWS ADHD LIKE BEHAVIOURAL PHENOTYPES**

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In RIKEN mouse ENU-mutagenesis project, we are carrying out behavioral screening to develop novel models for psychiatric disorders. In dominant phenotype screening, a mutant M-174 exhibiting hyper-locomotor activity in the open-field test was isolated from G1 population. In our linkage analysis, the phenotype was mapped to proximal region of chromosome 2 in which *Grin1* resides as a strong candidate gene for the mutation. We identified a non-synonymous missense mutation of C to T in the intracellular C0 region of *Grin1*. In detailed analysis of M-174 homozygous embryos, we found increased and prolonged calcium influx induced by NMDA stimulation in primary cultured cortical neuron, as compared with wild-type embryos. Moreover, sensitivity to administration of an NMDAR antagonist MK-801 and sensory stimulations via upper central nervous system (CNS) were changed in heterozygotes of M-174. These results indicate that the missense mutation in the C0 region of *Grin1* is responsible for the phenotype of M-174 mutant. Further analysis revealed that M-174 heterozygotes show attention deficit hyperactivity disorder (ADHD)-like phenotype, such as shorter duration of attention, abnormal reaction to methylphenidate (MPH) administration and a mild deficit in motor coordination. Thus, this study suggests that altered NMDAR function causes ADHD-related behavioral abnormalities in mice.

P121**MUTATION OF THE NADPH OXIDASE COMPONENT, P22PHOX/NMF333, RESULTS IN BOTH IMMUNOLOGICAL AND VESTIBULAR DEFICITS**

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The production of reactive oxygen species (ROS) by NADPH oxidases is a well-characterized system in phagocytic immune cells, where the production of ROS is required for the killing of ingested microorganisms. The immune NADPH oxidase consists of six components including gp91phox (Cybb/Nox2), p22phox (Cyba), p67phox (Ncf2), p47phox (Ncf1), p40phox (Ncf4), and Rac1/2. Defects in any of a number of these genes result in Chronic Granulomatous Disease (CGD), a life-threatening human condition characterized by recurrent bacterial and fungal infections. Recently, it has been shown that the founding member of the NADPH oxidase system (Nox2) is part of a larger family of related genes, including Nox1 and Nox3 through Nox5. Recently, our studies of head tilt (het) and head slant (hslt) mutant mice have demonstrated critical roles for Nox3 and Nox1 (a p47phox paralog), respectively, in the development and function of the vestibular system. Here, we describe the vestibular mutant nmf333 and show that the mutant phenotype results from a missense mutation in a transmembrane domain of p22phox/Cyba. Thus, nmf333 represents a third NADPH oxidase component critical for normal vestibular development and function. Because p22phox/Cyba is shared with the NADPH oxidase of circulating immune cells, nmf333 also represents a mouse model of Cytochrome B-negative, autosomal recessive, chronic granulomatous disease (OMIM #233690).

P122**ANALYSES ON LEARNING AND MEMORY IN MICE CARRYING mtDNA WITH A LARGE-SCALE DELETION.**

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Mitochondria possess multiple copies of mitochondrial DNA (mtDNA) that encodes 13 subunits essential for mitochondrial respiratory function. Accumulation of mtDNA with pathogenic mutations induces mitochondrial respiration deficiencies, resulting in mitochondrial diseases. Previously, we have generated mice model for mitochondrial disease (mito-miceD), which carry heteroplasmic mtDNAs with pathogenic 4696-bp deletion (DmtDNA). The several clinical phenotypes similar to mitochondrial diseases were expressed by accumulated DmtDNAs, because DmtDNAs are able to accumulate the threshold to induce mitochondrial respiration deficiencies with replication advantage. Recently, it has been reported the relationship between mutated mtDNAs and neurological disorders. Moreover, it is known that learning disability is one of main clinical phenotypes of mitochondrial diseases. However, there are no detail analyses to evaluate the role of mitochondrial respiration in brain functions, so that we performed spatial learning and memory task using mito-miceD.

To assess the ability of spatial learning and memory, we used Barnes circular maze. Mito-miceD with high loads of DmtDNA (mito-miceDH) decreased latency, errors, and distance to enter the escape hole with daily training similar to mito-miceD with low loads of DmtDNA (mito-miceDL), indicating that pathogenic DmtDNAs were not affect their learning abilities. To test the retention of long-term memory, we performed the probe-test at 36 days after training. The mito-miceDH showed a significant decrease in the retention of memory for a target hole compared with the mito-miceDL. These results suggest that accumulation of DmtDNAs and the resultant mitochondrial respiration defects is preferentially associated with abnormalities of long-term memory.

P123**SYSTEMATIC ANALYSIS OF BEHAVIORAL TRAITS USING CONSOMIC MOUSE STRAINS ESTABLISHED FROM C57BL/6J AND WILD-DERIVED MSM**

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Much of the genetic variation that underlies most behavioral traits is complex and is regulated by loci that have quantitative effect on the phenotype. We aimed to reveal those genetic mechanisms underlying individual divergence of behavior by using consomic strains (CSSs) of mouse established from C57BL/6J and MSM. By examining a panel of CSSs on many behavioral traits, such as spontaneous activity, anxiety-like behavior, and pain sensitivity, we systematically mapped the chromosomes that have a locus or loci affecting those phenotypes. To dissect complex traits into fine genetic elements, we focused on one strain B6-17MSM, which have substituted chromosome 17 from MSM. B6-17MSM showed reduced novelty-induced activity and increased risk-assessment behavior compared to C57BL/6J. They also showed increased fear memory in the fear conditioning test. Thus, it was expected that there are genetic locus/loci associated with emotionality on the chromosome 17. In addition, we found that B6-17MSM had "hydrocephalus-like" enlarged brain ventricle size. To identify genetic loci related to those phenotypes, we established a series of congenic mouse strains of B6-17MSM. Analysis of congenic strains successfully revealed two novel genetic loci for the brain ventricle size in the proximal region of chromosome 17. In contrast, there are multiple loci for the behaviors associated with novelty-induced activity and two genetic loci for risk-assessment. We focused on one locus around telomeric region (6.5Mb), which had as strong effect on the emotionality-related traits as B6-17MSM but independent from hydrocephalus phenotype, and are now trying to narrow this locus down to identify the gene.

P124**MODELING PARKINSON'S DISEASE IN MICE**

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Parkinson's disease (PD) is the second most common neurodegenerative disease in humans. Diagnosis of the disease is possible only upon the appearance of motor symptoms, which requires loss of a majority of dopaminergic neurons in the substantia nigra. Dopaminergic neurons are not the first to be affected in PD, but earlier symptoms caused by affected neurons in brainstem and the olfactory system are too non-specific for diagnosis. Thus the early stages of PD cannot be studied in humans, and an animal model that affects the appropriate brain regions in the proper progression is needed. While most cases of PD are sporadic and of unknown etiology, rare genetic forms occur. Mutations in α -synuclein were the first identified as causing PD. Transgenic mice expressing mutant human α -synuclein under the control of heterologous promoters have been generated to model PD, but have developed disease in regions of the nervous system not affected in PD. We have used a human α -synuclein PAC to generate transgenic mice that express the transgene in the same pattern as endogenous mouse α -synuclein. Aged wild type PAC mice develop late onset changes in the nigra and the striatum, but do not develop the characteristic Lewy body pathology seen in PD. To lower the age of onset, the α -synuclein PAC has been mutated to a form that in humans causes PD in the 40's rather than the 70's. Cohorts of mice carrying this mutant PAC transgene are now being aged and monitored for onset of brainstem and nigrostriatal phenotypes.

P125**mRNA-LIKE NON-CODING RNAs INVOLVED IN CELL FATE SPECIFICATION OF NEURONAL STEM CELLS**

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The progress of genomic DNA sequencing and comprehensive cDNA collection in mammalian model organisms have revealed the intricacies of genomic organization and the presence of various mRNA-like non-coding transcripts (mIncRNAs). Most of the functions of mIncRNAs remain to be elucidated, while vast numbers of mIncRNAs and their vast variety of genomic organizations have implied that they might have some biological significance.

During development of the central nervous system, neurons, astrocytes and oligodendrocytes are generated from a common progenitor, neuronal stem cell. It has been shown that some proneural genes are involved in determination of cellular fates of neuronal stem cells into each cell type. Searching mouse cDNA databases showed that several mIncRNAs are transcribed from the vicinity of the genomic regions of some of proneural genes. We tested if these proneural gene-related mIncRNAs are involved in specification of cellular fates of neuronal stem cells. Quantitative RT-PCR experiments detected the changes in transcription levels of some of the mIncRNAs examined in differentiation process induced by LIF and BMP2. *In situ* hybridization experiments showed that most of the mIncRNAs tested are actually expressed in mouse brains with various expression patterns and at varied levels. Overexpression of mIncRNAs in cultured neuronal stem cells suggested that at least a few of the mIncRNAs examined might have certain functional roles in cellular fate determination of neuronal stem cells. These results suggest that some of the proneural gene-related mIncRNAs have biological significance by taking parts in the molecular network underlying differentiation of neuronal stem cells.

P126**TRANSCRIPTOME PROFILES OF A SMALL NUMBER OF IDENTIFIED NEURONS BY COMBINING LASER CAPTURE MICRODISSECTION AND nanoCAGE-ILLUMINA-SOLEXA TECHNOLOGY**

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Recent advances in studying the mammalian transcriptome arised new questions about how genes are organized and what is the function of noncoding RNAs. Furthermore, the discovery of large amounts of polyA- transcripts proved that a portion of the transcriptome has still to be characterized. The complex anatomo-functional organization of the brain has prevented a comprehensive analysis of the transcriptional landscape of this tissue. New techniques must be developed to approach neuronal heterogeneity. In this study we combined Laser Capture Microdissection (LCM) and Cap Analysis of Gene Expression (CAGE) to describe expressed genes and map their transcription start sites (TSS) in specific populations of cells. Specific neurons were identified by the expression of Green Fluorescent Protein driven by a cell-type specific promoter in transgenic mice. High-quality RNAs were purified from 1000-2500 cells collected by LCM. We adapted the CAGE technique to analyze limiting amounts of RNAs (nanoCAGE). We took advantage of the cap-switching properties of the reverse transcriptase to specifically tag the 5'end of transcripts with a sequence containing a class III restriction site for EcoP15I. By creating 32bp 5'tags, we considerably improved the TSS mapping rate on the genome. A semi-suppressive PCR strategy was used to prevent primer dimers formation. The use of random priming in the 1st strand synthesis allowed to capture poly(A)- RNAs. 5'tags were sequenced with Illumina-Solexa platform. Here we show that this new nanoCAGE technology ensures a true high-throughput coverage of the transcriptome of a small number of identified neurons.

P127**GENETIC DETERMINATION OF LEPTIN, ADIPONECTIN, INSULIN AND IGF-1 LEVELS IN THE BERLIN FAT MOUSE INBRED 860 LINE**

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The research on endocrine factors that are involved in regulation of body weight and fat deposition contributes to our understanding of the complexity of polygenic obesity. To investigate the role of hormones we use the high fatness selected Berlin Fat Mouse Inbred 860 (BFMI860) line as a model for polygenic obesity. At 10 weeks BFMI860 males exhibit 5.1 fold increased body fat percentage in comparison to mice of the unselected control line C57BL/6 (B6). Under a normal maintenance diet, BFMI860 males showed about 10 times higher leptin levels than B6 males. Adiponectin levels were 1.5 times lower in BFMI860 animals compared to B6. Insulin concentrations were 17 times and IGF-1 concentrations 1.5 times higher in BFMI860 compared to B6 mice, respectively. In response to a high fat diet, leptin and insulin concentrations increased in both lines (2.6 and 10 fold for BFMI860, both 3 fold in B6, respectively) while adiponectin levels declined only in the line BFMI860. IGF-1 did not change in response to diets, neither in B6 nor in BFMI860 mice. The BFMI860 animals show an endocrine profile typical for the obesity status. That provides an indication of a change in the regulation of body weight and fat deposition. To identify chromosomal regions influencing these hormones we will perform a linkage analysis with animals of the cross BFMI860xB6.

P128**CONSOMIC STRAINS AS A TOOL FOR DISSECTION OF REGULATORY NETWORKS**

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Genetically determined variations between individuals, such as RNA expression levels, patterns of alternative splicing, protein abundance and modification, resistance to a particular infection or predisposition to particular tumors, are based on complex biological processes controlled by networks of multiple genes and identified as quantitative trait loci (QTLs). The activity of these genetic factors is under the control of regulatory networks that are still poorly defined and understood. Consomic strains (also named chromosome substitution strains) constitute the latest addition to the mouse genetic resources aimed at upgrading the genetic analysis of quantitative traits. We have developed a set of inter-species mouse consomic strains by using the PWD/Ph inbred strain of *Mus musculus musculus* origin as the chromosome donor and the C57BL/6J strain (mostly *Mus musculus domesticus*) as the genetic background strain. The two progenitor strains C57BL/6J and PWD/Ph are roughly 500 000 years apart in evolution, accounting for a large number of genetic differences (approx. 1% as compared to approx. 0.1% in humans). We will report our pilot gene expression profiling experiments on RNA from the whole brains with the Illumina and Affymetrix Mouse expression platforms. The analysis of individual consomic strains allowed us to identify differentially cis-regulated genes, located on the introgressed chromosome from the trans-regulated genes localized in other sites of the genome. We will also show that by using the rQTL and QTL reaper programs we could map the regulatory factors on the panel of intercross F2 animals generated from the (C57BL/6 x consomic)F1 parents.

P129**EFFECTS OF COMPLETE DEFICIENCY AND C-TERMINAL DELETION OF ADAMTS13 ON HEMOSTATIC FUNCTION IN MICE**

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ADAMTS13 is a multidomain metalloprotease that regulates platelet aggregation through cleavage of von Willebrand factor (VWF) multimers. Genetic or acquired deficiency in ADAMTS13 leads to thrombotic thrombocytopenic purpura (TTP), a condition characterized by thrombocytopenia, hemolytic anemia, and microvascular thrombosis. In this study, we generated and characterized Adamts13-knockout mice (KO) and Adamts13-congenic mice lacking the C-terminal domains of ADAMTS13 (CG). Both KO and CG were viable and fertile. In KO, unusually large VWF multimers were observed in the plasma. Thrombogenesis on immobilized collagen under flow and thrombocytopenia induced by a collagen plus epinephrine infusion were significantly promoted in KO than in wild-type mice (WT). However, hematological and histological analyses failed to detect any signs of TTP in KO. These observations and accumulating clinical information on TTP patients suggest that ADAMTS13 deficiency alone is not sufficient to cause TTP. KO may be useful for elucidating the genetic and environmental triggers for TTP. In contrast to KO, CG maintained the ADAMTS13 activity and normal VWF-multimer distribution in the plasma, and did not show an enhanced thrombogenesis under flow. Then, we employed thrombogenic challenge tests. Intravenous infusion of DDAVP increased the large VWF multimers in the plasma, and the multimers were decreased more slowly in CG than in WT. The blood collected from CG after DDAVP infusion exhibited more enhanced thrombogenesis under flow compared with the blood from WT. Thrombocytopenia induced by a collagen plus epinephrine infusion was most remarkable in KO, and significantly more in CG than in WT. These results suggest that the C-terminal domains of ADAMTS13 contributes to the processing of large VWF multimers in the blood vessels, and that the importance of these domains become obvious when vascular damage is induced.

P130**GENOME-WIDE SEARCH FOR GENES WHICH MODULATE INFLAMMATORY ARTHRITIS CAUSED BY ALI18 MUTATION IN MICE**

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Many of inflammatory diseases including inflammatory arthritis are multifactorial bases, which consist of complex combinations of environmental and genetic factors. These hamper identification of genetic components of the diseases. In the Munich screen of ENU induced mutant mice, we have isolated and characterized the Ali18 mutant mice, which exhibit inflammatory arthritis and dermatitis in peripheral limbs; the Ali18 is a semidominant mutation and was mapped to Chromosome 4 (Mammalian Genome 17, 915-). In the course of genetic mapping, we found phenotypes of Ali18/+ mice were completely suppressed in hybrid genetic backgrounds. To dissect genetic components of the Ali18 mediated pathway, we conducted an autosomal genome scan using 174 N2 (backcross to Ali18/Ali18) and 267 F2 animals between C3HeB/FeJ-Ali18/Ali18 and C57BL/6J parental strains. We selected two parameters, clinical scores of paw swelling and plasma IgE levels, for phenotyping and 153 polymorphic SNP markers for genotyping. In N2 populations, there are two suggestive quantitative trait loci (QTLs) of arthritis scores on Chromosomes 1 and 15. Involvement of the both chromosomes were detected also in F2 populations: two QTL interactions, Chromosomes 1 x 10 and 15 x 6 with maximum LOD score of 6.25 and 4.80, respectively, were identified. Because no significant QTLs about IgE levels were detected, IgE upregulation may not be the first trigger of the inflammation. Higher resolution of the QTLs by means of congenic analysis, candidate approach, and expression profiling might contribute to dissect complexities of inflammatory diseases in human populations.

P131**REVERSE GENETIC STUDIES ON PATHOGENESES OF MITOCHONDRIA-RELATED MALE INFERTILITY**

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About 15% of human couples are affected by infertility, and about half of these cases of infertility can be attributed to men, through low sperm numbers (oligospermia) or low motility (asthenozoospermia). Sperm number and motility are the major determinants of male infertility in human, but little is still known about the genetic evidence and pathophysiological mechanisms of male infertility. Because sperm motility is dependent upon energy supply and mitochondrial genome (mtDNA) mutations are identified in patients with fertility problems, there is a possibility that mitochondrial respiration defects contribute to male infertility. To address this possibility, we used a trans-mitochondrial mouse model (mito-mice) carrying wild type mtDNA and mutant mtDNA with a pathogenic 4696-bp deletion (del-mtDNA). Here we show that mitochondrial respiration defects due to accumulation of the del-mtDNA induced not only asthenozoospermia but also oligospermia, leading to male infertility. Testes of the infertile mito-mice showed meiotic arrest at the zygotene stage and enhanced cell death, suggesting the existence of energy-dependent checkpoint in the meiotic process. Thus, our results directly demonstrate for the first time that normal mitochondrial respiration is essential for mammalian spermatogenesis, and that its defects due to accumulated mutant mtDNAs cause for oligospermia and asthenozoospermia, leading to male infertility.

P132

A COMPREHENSIVE QTL ANALYSIS ON THE SERUM CHOLESTEROL LEVEL BEFORE AND AFTER A HIGH-CHOLESTEROL DIET IN SHRSP

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[Objective] The stroke-prone spontaneously hypertensive rat (SHRSP) showed an exaggerated response to a high-fat high-cholesterol (HFC) diet, and the resulting reactive hypercholesterolemia, which was suggested to exacerbate the atherogenic process in this rat. We thus performed a QTL analysis on the serum cholesterol level of SHRSP before and after the HFC diet to identify the genetic mechanisms of the reactive hypercholesterolemia in this strain. **[Method]** Three hundred and fifty-eight F2 rats between SHRSP and Wistar-Kyoto rat (WKY) were employed in the study. The serum cholesterol [total cholesterol (TC), VLDL-C, LDL-C and HDL-C] were measured before and after the 2-weeks of feeding the HFC diet. **[Results]** Multiple QTLs for the basal TC level were identified on chromosome 1 and 5, while those for the post-dietary TC level were on chromosome 7, 15 and 16. The cholesterol QTLs before and after HFC diet did not overlap with one another, implying that the involved metabolic processes were considerably different between the two conditions. Supporting this, VLDL-C and LDL-C were the major components of the post-dietary TC, while the basal TC consisted mainly of HDL-C. A substantial difference of the QTLs between the male and the female was observed, especially after the HFC diet. The QTL on chromosome 15 had an inverse effect on the cholesterol level, suggesting that the congenic substitution of the SHRSP fragment with that of WKY could induce a greater cholesterol level in SHRSP. **[Conclusions]** This observation is significant to establish a new model for atherosclerosis with hypertension in rats.

P133

FINE MAPPING OF ADIPOSITY QTL ON MOUSE PROXIMAL CHROMOSOME 2 BY SUBCONGENIC ANALYSIS

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Many QTLs affecting growth, adiposity and related traits have been reported on mouse chromosome 2. However, mapping resolution of most QTLs studies are too poor to find responsible genes. Thus, it is imperative to confirm the positions of identified QTLs and to fine map them. Subcongenic analysis is well known to be a powerful method for fine mapping QTL because it allows multiple tests for phenotypic effects on a uniform genetic background expect for the QTL region. In our previous study we developed the congenic strain, B6.Cg-Pbwg1, with a 44.1-Mb donor region from the Philippines wild mouse (*Mus musculus castaneus*) onto the C57BL/6J genetic background. QTL analysis in an intercross between B6.Cg-Pbwg1 and C57BL/6J showed that two adiposity QTLs are closely linked and both castaneus alleles exert negative effect on adiposity (Ishikawa et al., *Mamm Genome*, 2007). In this study we developed 14 overlapping subcongenic strains from B6.Cg-Pbwg1 to resolve the linked QTLs into individual loci. Phenotypic analyses of several overlapping subcongenic strains revealed that at least one adiposity QTL is probably located within a small region of about 5-Mb, containing 12 known and predicted genes. Now we are performing expression analysis of the genes within the region by Affymetrix Gene Chip and RT-qPCR. The results will be shown in this conference.

P134**MEIOTIC ARREST IN MALES OF THE B6.ChrXMSM CONSOMIC STRAIN**

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Small testis is the most prominent phenotype of mouse interspecific and intersubspecific hybrids. The complete loss or decreased number of mature sperms in the hybrid males directly influence on the fertility. We have established a consomic strain B6.ChrXMSM, which has X chromosome of Japanese wild mouse (*M. m. molossinus*)-derived inbred strain MSM/Ms on the genetic background of a standard inbred strain C57BL/6J (predominantly *M. m. domesticus*). Males of the consomic strain are sterile and show reduced testis size. Our and other studies previously reported that the meiotic arrest is observed at pachytene stage in such small testes. During this stage, heteromorphic sex chromosomes make synapsis in a pseudoautosomal region at their distal ends and undergo transcriptional inactivation, termed meiotic sex chromosome inactivation (MSCI), by remodeling into heterochromatin, thereby forming the XY body. We therefore investigated the formation of XY body in B6.ChrXMSM testes. Intriguingly, considerable number of spermatocytes showed not only defective formation of the XY bodies but also incomplete synapsis between sister-chromatids of the autosomes. From our observation and the generality of pachytene arrest observed in hybrid males, we infer that the pachytene checkpoint to monitor the proper synapsis and XY body formation plays a crucial role to discriminate the genome from other species or subspecies, which maintains the reproductive barriers.

P135**GENETIC SUSCEPTIBILITY TO PORPHYROMONAS GINGIVALIS-INDUCED ALVEOLAR BONE LOSS IN MICE**

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Periodontal diseases are chronic inflammatory diseases which result in the breakdown of the supporting tissues of the teeth, including resorption of the alveolar bone of the jaw. Previous Epidemiological studies have suggested that susceptibility to chronic periodontitis is controlled by genetic factors of the host. For understanding and mapping the genetic factors underlying host susceptibility to the disease, we used a murine model in which alveolar bone loss is induced by oral infection with perio-pathogenic bacteria, and we determined whether inbred strains of mice differ in their susceptibility to alveolar bone loss. Age matched male and female mice of different inbred strains (BALB/CJ, DBA/2J, C57BL/6J and A/J) were orally infected with *Porphyromonas gingivalis* and *Fusobacterium Nucleatum*, gram negative bacteria associated with human adult periodontal disease (n=10 for each strain). The infection was repeated three times at 2 day intervals. Six weeks following the final infection, the maxillary jaws were harvested and analyzed for alveolar bone loss using microCT technique. Our results have shown that there is no significant difference on alveolar bone loss between male and female of all tested strains. BALB/CJ mice were highly susceptible while DBA/2J, C57BL/6J and A/J were much more resistant. These results open the possibility of exploiting the oral infection mice model to identify loci important for host susceptibility and resistance to periodontal disease. Currently, we are at the process of performing genome-wide search for mapping quantitative trait loci (QTL) associated with host susceptibility to periodontitis in F2 (BALB/c x C57BL/6) resource population.

P136

GENETIC DISSECTION OF QUANTITATIVE TRAIT LOCI AFFECTING MULTIPLE PHENOTYPES IN HETEROGENEOUS STOCK MICE

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In 2006 we reported the detection and fine-mapping of 843 QTLs affecting 97 complex disease traits in 2000 heterogeneous stock mice (Valdar et al, 2006, Nature Genetics), the largest study of its kind at that time. Since then we have performed extensive analysis of the implicated QTL regions, enriched the data set with expression data for 500 animals and found evidence for pleiotropic networks of QTLs, co-expression networks associated with QTL sets, and strong relationships between co-localizing cis-eQTLs and QTLs. Here we report these findings and describe how they contribute to the functional characterization of fine-mapped QTLs and the molecular identification of the causal genetic variants.

References:

Valdar W, Solberg LC, Gaugier D, Burnett S, Klenerman P, Cookson WO, Taylor M, Rawlins JNP, Mott R, Flint J (2006) Genome-wide genetic association of complex traits in outbred mice. Nature Genetics 38(8):879-87

P137

EXPRESSION OF PLA2G2A PREVENTS TUMORIGENESIS IN AZOXYMETHANE-TREATED C57BL/6 MICE; GENE EXPRESSION STUDIES REVEAL PLA2G2A TARGET GENES IN MOUSE COLON

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The secretory phospholipase Pla2g2a is a part of the Mom1 locus that provides resistance to intestinal tumorigenesis in the ApcMin/+ mouse model, where this protection is most pronounced in the large intestine. We tested whether expression of a Pla2g2a transgene could also provide protection against colon tumors induced by treatment of C57BL/6J mice with the DNA alkylating agent, azoxymethane (AOM). In 6-week-old mice treated with AOM at a dosage of 10 mg/kg body weight for 6 consecutive weeks and subsequently aged to 240 and 360 days we found that expression of Pla2g2a strongly inhibited colon tumorigenesis in both male and female mice. A novel finding is that AOM also induced tumors in the small intestine of C57BL/6J mice, principally in the duodenum, a phenotype that also was attenuated by expression of Pla2g2a. Immunohistochemical analysis showed that these duodenal tumors demonstrated upregulation of β -catenin protein. Finally, we conducted gene expression studies to discover genes differentially expressed in response to Pla2g2a. Clusters of genes involved in inflammation and microbial defense (Defb11, Syvn1, Adam11, Ifng, IL17ra, Cx3cl1), cell signaling and cell cycle (Rgs3, Zfyve9, Taok1, Ephb2, Ncor2), transactivation (Runx1, Mier2, Hoxb9, Lhx1, Cdx2, Klf16), apoptosis and mitochondrial function (Bax, Vdac1, Map3k10, Birc6, Ak2, Arl6ip5), lipid and energy metabolism (Sreb2, B3galt1, Ehbp1, Neto1, Pocr) and DNA repair (Ercc2, Parp1) were identified as Pla2g2a target genes. Overall, our results confirm that Pla2g2a prevents intestinal tumors independent of germline mutations in Apc and we have identified Pla2g2a target genes of potential therapeutic value for the treatment of human colorectal cancer.

P138**IDENTIFICATION OF A NEONATAL GROWTH GENE CANDIDATE USING INTEGRATIVE GENOMICS**

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Inbred strains of mice differ greatly in their maternal performance and neonatal growth, and serve as good models for identification of putative candidate genes. Recent availability of whole genome SNP genotypes mouse strains provides a powerful system for complex trait analysis. Whole genome SNP association analysis of reproductive performance phenotypes in 24 strains of mice using data from public databases was used to identify candidate regions. A strain of mice (QSi5), developed at the University of Sydney, which has a superior phenotype when compared to other mouse strains, was used for gene expression profiles and compared with CBA mice to help identify the genes responsible for neonatal growth. RNA isolated from mammary tissues collected at peak lactation (Day 9) from 5 animals in each strain were hybridised to Affymetrix MOE 430 arrays. The genes showing differential expression were compared with other lactation and QTL datasets, and in particular a QTL on mouse chromosome 9. Integration of QTL data with mouse strain genotypes, excluding regions that were identical by descent, and incorporating maternal gene expression profiles, reduced the potential candidates to a sole gene, Neo1. A survey of the literature on this gene revealed that Neo1 is expressed in cap cells within the mammary gland and has a role in morphogenesis. Thus, we have identified Neo1 as a maternal candidate gene responsible for the superior neonatal growth phenotype of QSi5 strain of mice by adopting an integrative genomics approach. The gene is currently the subject of ongoing analysis.

P139**GENETIC DISSECTION OF AN ICR CLOSED COLONY OF THE MOUSE**

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It has been known that closed colonies have provided animal models for human diseases, i.e., NOD. The authors have a hypothesis that spontaneously occurred mutations are accumulated in colonies. To demonstrate this, we have performed mating experiments to find male mice carrying recessive mutation(s).

Male mice randomly selected from an ICR colony were subjected to this study. An ICR male mouse was mated with a DBA/2Jcl female to obtain F₁ mice. Four F₁ females randomly selected were backcrossed to the ICR male mouse (their father). At least thirty backcross mice were obtained from each female. Their phenotypes were observed at about one month after birth.

Eighty-one male mice were mated with the female mice, but twelve ICR male mice were infertile. We observed that eighteen (26.1%) of sixty-nine male mice carried autosomal recessive gene(s) responsible for inheritable anomalies. They were as follows; 1)dwarf (No.8 and No.79), 2)hind limb paralysis (No.20), 3)wobbling (No.25, No.30, No.63 and No.74), 4)hydrocephalus (No.32, No.54 and No.61), 5)circling (No.39), 6)small testis (No.43, No.57 and No.68), 7)abnormal kidney (No.43), 8)rigidity (No.48), 9)aplasia of eye lid/hind limb fingers (No.52 and No.78), 10)heterotaxia (No.62), 11)shiver (No.79) and 12)dystrophy (No.79).

We successfully demonstrated that recessive spontaneous mutations have occurred and accumulated in the colony. The results obtained in this study strongly suggested that spontaneous mutations will be observed in not only mouse colonies but also rat colonies. We should pay special attention to such mutations which may bring confusing results, when closed colonies are used in toxicology studies.

P140**HIGH-RESOLUTION FULL COLOR DIGITAL MOUSE ANATOMY BY THREE-DIMENSIONAL INTERNAL STRUCTURE MICROSCOPY**

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The anatomical atlas of mammalian species is one of the essential information to correlate the structures and gene functions of the organism. We have created high-resolution full-color volume data of genus *Mus* by using the three-dimensional internal structure microscopy (3D-ISM). Several mouse strains derived from distinct origins including C57BL/6 (standard laboratory inbred: *Mus musculus domesticus*), PWK (*Mus musculus musculus*), MSM (*Mus musculus molossinus*), HMI (*Mus musculus castaneus*), ZBN (*Mus spicilegus*), *M. spretus* (*Mus spretus*) and Car (*Mus caroli*) were used at 8 weeks of age. The mice were euthanized by excess anesthesia, treated for hair removal, immediately placed in -80 degrees C freezer, embedded in blue-colored OCT compound and serially sectioned by 3D-ISM system. Full-color serial images of the whole body at resolution of 25 mm x 25 mm x 20 mm were imported to Volume Computing Aided Testing (V-CAT) software and reconstructed as 3D-images composed of the volume data. Through the color volume rendering viewer the internal structures of the body could be observed in full-color at any desired sectional plane. Our digital data may provide a useful guide to the anatomy of genus *Mus* and high-quality digital template for measuring anatomical structures *in silico* without further sacrifice of the animals. We also plan to use these data to correlate the structures and gene functions in the mouse.

P141**LYMPHOMA SUSCEPTIBILITY AFFECTS THE DEVELOPMENT OF CLONALLY GROWING PRE-LYMPHOMA CELLS**

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Center for Transdisciplinary Research

Population-based epidemiological studies suggest that a high proportion of cancers arise in a susceptible subpopulation that carry low-penetrance variant alleles. One major model for polygenic predisposition is the common variant-common disease model that underlies disease predisposition in humans and perhaps in mice. BALB/c and C57BL/6(B6) mouse strains are highly susceptible to radiogenic thymic lymphomas whereas MSM and C3H strains are resistant. Our previous genetic study using BALB/c and MSM strains localized a susceptibility locus within 4 Mb region on chromosome 4 and identified *Mtf-1* as a candidate susceptibility gene to radiogenic lymphomas. The two different *Mtf-1* alleles, BALB/c encoding the serine-type MTF-1 and MSM encoding the proline-type, exhibited distinct transcriptional activation and responses to ionizing radiation (IR). To reveal the mechanism for this susceptibility, we examined effects of g-ray on pre-lymphoma cells *in vivo*. Here we show the detection of clonally growing large thymocytes in thymuses at as early as 5 weeks after irradiation. Interestingly, some thymuses exhibited genetic changes such as allelic loss at *Bcl11b*, indicating the start of clonal expansion of pre-lymphoma cells at a very early stage. We also show that proliferating large thymocytes exhibiting higher ROS levels were more numerous in the *Mtf-1* susceptible mice than the resistant mice, although such tendency was not found in mice lacking one allele of *Bcl11b* tumor suppressor gene. This high retention of the large thymocytes as a compensatory proliferation response to irradiation may be a cause to augment the development of pre-lymphoma cells leading to thymic lymphomas.

P142**FINE MAPPING OF AHL3 AFFECTING BOTH AGE-RELATED AND NOISE-INDUCED HEARING LOSS**

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Different susceptibility to noise-induced hearing loss (NIHL) has been suggested in human populations. When equal noise exposures are given, some susceptible individuals show a greater permanent threshold shift (PTS) in auditory brainstem response (ABR) threshold measurements than others. The range of individual susceptibility is probably affected by genetic variation, but no contributing loci have been identified. One approach for unraveling the genetic basis of NIHL is to use mouse models, and MSM mice exhibit no PTS after noise exposures whereas C57BL/6(B6) mice show PTS. Age-related hearing loss (AHL) is also known to be a complex trait and genetic basis for susceptibility to NIHL and AHL appears to be correlated. An *ahl* allele of the *Cdh23* gene, a mutation of which causes a congenital deafness in mice and humans, affects both AHL and NIHL. *Cdh23* encodes a cadherin molecule thought to be a component of the tip links joining adjacent stereocilia at the top of sensory hair cells. We previously mapped another AHL locus, *Ahl3*, in the vicinity of *D17Mit119* on mouse chromosome 17 using a congenic strain of B6 background substituting the B6 chromosome 17 with the MSM-derived one (B6-Chr17^{MSM}). This paper presents fine mapping of the *Ahl3* locus using different sets of congenic mice, which localizes *Ahl3* within a 14-Mb region on mouse chromosome 17. Furthermore, we show resistance to NIHL of the congenic mice carrying the 14-Mb region, indicating the role of *Ahl3* in susceptibility to NIHL as well.

P143**GENOMIC COPY NUMBER AND EXPRESSION VARIATION WITHIN THE C57BL/6J INBRED MOUSE STRAIN**

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The C57BL/6J inbred strain has been maintained for over 70 years through more than 200 generations of brother sister mating. It is one of the most widely used animal models for biomedical research and individual mice within the strain are often assumed to be genetically identical. Using a SNP genotyping panel, we assessed if copy number variations (CNVs) could be detected within the C57BL/6J strain by comparing the relative allele frequencies in first generation (F1) progeny of C57BL/6J mice. With a combination of sequencing, quantitative PCR, breeding, and array comparative genomic hybridization (CGH), we confirmed the presence of 2 CNVs. Both CNVs span genes encoded on Chromosome 19 and quantitative RT-PCR demonstrated that they result in altered expression of the insulin degrading enzyme (*Ide*) and fibroblast growth factor binding protein 3 (*Fgfbp3*) genes. FISH and pedigree analysis showed that the extra copy of *Ide* present in some C57BL/6J animals is in tight linkage with the known Chr 19 location. Analysis of 49 different C57BL/6J breeders revealed that 63% of mice from the Jackson laboratory colony were heterozygous for the CNV spanning *Ide*. The identification of 2 CNVs in the small portion of the genome screened (<0.01%) demonstrates that individual mice of highly inbred strains are not isogenic and suggests that many other CNVs are likely to be segregating within C57BL/6J as well as other carefully maintained inbred strains. These differences can influence interpretations of physiological, biomedical, and behavioral experiments and be exploited to model CNVs apparent in the human genome.

P144**FINE MAPPING OF LOCI THAT MODIFY SUSCEPTIBILITY TO N-METHYL-N-NITROSOUREA (MNU)-INDUCED T-CELL TUMORS**

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Most cancers in humans result from exposure to environmental carcinogens and multiple genes modify the response of individuals to exposure. The overall susceptibility of an individual is determined by the combined effects of both susceptibility and resistance genes. The mapping and isolation of such low penetrance genes in humans is complicated by the multiplicity of unlinked loci involved. Animal models are useful experimental tools for identifying and characterizing such genetic factors. N-methyl-N-nitrosourea (MNU) induces T-cell lymphomas in mice and susceptibility is a multigenic trait. AKR/J and DBA/2 mice have a higher incidence and shorter latency than most other inbred mouse strains that have been tested. Using quantitative trait locus (QTL) analysis, we, and others, previously mapped MNU susceptibility loci to chromosomes (chr) 4 (Tlag2) and 7 (Tlag1) in crosses of AKR with resistant C57L mice and to chr 1 (Tli1) in crosses of AKR with resistant BALB/c mice. To narrow the map location of each locus we used a multiple cross mapping strategy combined with interval-specific haplotype analysis which takes advantage of the mosaic structure of variation among the genomes of common laboratory mouse strains. Sensitive AKR and DBA/2 mice were intercrossed with resistant C57L and BALB/c mice and susceptibility to MNU induction of lymphomas was determined for mice from each F2 population. The allelic state of each strain was determined for each locus and haplotypes were analyzed for the new QTL intervals to identify chromosomal regions with genotypes that correlated with the allelic states of the strains.

P145**ANALYSIS OF Skts-fp1 LOCUS FOR SKIN TUMOR SUSCEPTIBILITY IN DOMINANT RESISTANT BACKCROSS**Kyoko Fujiwara¹, Hiroki Nagase²¹Cancer Genetics, Roswell Park Cancer Institute, ²Advanced Research Institute for the Sciences and Humanities, Nihon Univ.

Cancer susceptibility is varied among mouse strains, which enable us to reveal genetic background differences which contribute to cancer development. The two-stage chemical skin carcinogenesis model [induced by dimethylbenz(a)anthracene (DMBA)/12-O-tetradecanoylphorbol-13-acetate (TPA)] has been analyzed extensively and some of the susceptible genes have been identified by this procedure. We reported previously that PWK is a dominant resistant for skin tumor when it is crossed with FVB, and significant linkage for skin tumor susceptibility was found on chromosome 4 by analyzing 178 of (FVBxPWK)F1 x FVB backcross. In the present study, we analyzed 530 backcrosses including 178 of previous backcross. Significant linkage Skts-fp1 was mapped between D4Mit111 and D4Mit308 (21.9-57.4 cM) on chromosome 4 with the maximum LOD score of 20. Among 80 of phenotype-driven congenic strains, which have been backcrossed to FVB strain in fifth generations, all of 4 mice with complete resistance for skin tumor development shared the PWK allele between D4Mit111 and D4Mit122 (21.9-56.0 cM). Analysis of loss of heterozygosity (LOH) in skin tumors originated from the backcross mice with heterozygous at Skts-fp1 to narrow down this locus. Nine out of 60 tumor samples showed LOH on the Skts-fp1, and 6 of them shared a common deletion of the PWK allele between marker D4Mit26 and D4Mit165 (42.5-44.5 cM). This result suggests that one of the candidate genes could be located within this interval. We will present data of allele-specific changes in expression and mutation of genes within the interval.

P146**Idd1a, Idd1b, Idd1c and Idd1d EXERT THEIR FUNCTIONS FROM 2 DAYS OLD THROUGH 14 DAYS OLD IN THE NEONATAL INDUCTION OF DIABETES BY DIABETOGENIC CD4+BDC2.5 T CELL CLONES**

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The NOD mouse develops autoimmune type 1 diabetes. The genes in the /MHC/ region are important in determining susceptibility to type 1 diabetes. Congenic lines of NOD with B10A.(R209) mice have shown that there are four diabetogenic, *Idd1a*, *Idd1b*, *Idd1c* and *Idd1d* genes in the region centromeric to the *Lmp2* gene on chromosome 17. To examine the function of the genes, we transferred 1×10^7 activated BDC2.5 T cell clones into 1- to 15-day-old G1(NOD-type), G3 (*Idd1a, b, c and d*) and C (*Idd1a and b*) line neonates and screened them for the development of diabetes twice a day. The BDC2.5 T cells were activated by islet b granules prior to the transfer. Both 1-day-old G1 (10/10) and G3 (7/7) recipients developed insulinitis and diabetes within 7 days after the transfer with no difference in the onset of diabetes. Line C and G3 recipients aged from 2 to 10 days, however, delayed the onset of diabetes significantly in comparison with the age-matched G1 recipients. Fifteen-day-old recipients of G1 (0/4) and G3 (0/5) did not develop diabetes after the transfer during the observation of 20 days. Twelve hours after the transfer into 5-day-old G1 and G3 recipients, CFSE-labeled BDC2.5 T cell clones were found in both the endocrine islet and exocrine acinar tissues of the pancreas but not in the spleen. The results suggest that *Idd1a*, *Idd1b* and *Idd1c* exert their functions from 2 days old through 14 days old in the neonatal induction of diabetes by diabetogenic CD4⁺ BDC2.5 T cell clones.

P147**SEARCH FOR GENES CONTRIBUTING TO COLON CANCER SUSCEPTIBILITY**

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Earlier studies to determine the genetic basis for carcinogen-based susceptibility to colon cancer involved a backcross that identified four genetic regions on Chrs 5, 12, 13 and 14. Congenic strains, C57BL.ICR, were constructed for these regions and each was confirmed to contain genes involved with susceptibility. Development of subcongenic strains has shown that on Chr 12 the gene(s) lie in an interval of 10.8 Mb containing 81 genes and ESTs and in Chr 14 they lie in an interval of 10.5 Mb, containing 106 genes and ESTs. Subcongenic strains have been made for each interval and are being tested for tumor susceptibility. Meantime chosen genes have been tested individually for expression and sequence differences. In a study of transcription differences between colons from ICR and B6, using Affymetrix chips, expression differences were found between 15 genes, two from the Chr 12 congenic interval and one from the Chr 5 interval. Twelve other genes were unlinked to the previously described congenic intervals. Proteomic analysis between the two strains showed two genes with polymorphisms and three others with expression differences. These results have eliminated a number of genes from consideration and suggest certain genes for further study. NIH grant CA115436.

P148**MORPHOMETRIC AND MOLECULAR ANALYSIS OF BXD RECOMBINANT INBRED STRAINS**

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Recombinant Inbred (RI) mouse strain panels enable both genetic association studies and the integration of multi-system, multi-investigator datasets due to their stable genetic architecture. As such, RI strains are a valuable model for the study of complex traits. Our long term goal is to dissect the genetic interactions that control adipose tissue mass due to its relationship with many disease processes, such as Type 2 diabetes. As a starting point, we collected and analyzed morphometric and phenotypic data on 41 strains of BXD (C57BL/6J X DBA/2J) RI mice, a panel selected for its size and its wealth of existing multiscale data for other traits. Fasting plasma glucose levels, body weight, organ (heart, kidney, liver, thymus, spleen) and fat pad weights were measured from adult male and female mice, 3-5 mice per strain. Adiposity index was calculated to reflect overall fatness. Existing genotype data and analytical tools resident within WebQTL (genenetwork.org) were used to screen for regions of the genome containing polymorphisms that co-segregated with each trait. Several genomic regions of interest were identified, particularly for adiposity. Parallel analysis of relevant molecular traits is underway. Genomic regions of interest and potential candidate genes within those regions will be presented.

P149**HYPOTHYROIDISM-INDUCED DEAFNESS IS ASSOCIATED WITH POOR INNERVATION, REDUCED POTASSIUM CHANNEL GENE EXPRESSION, AND GENETIC MODIFIERS**

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Congenital hypothyroidism causes permanent hearing deficits in human and mice, but the underlying mechanism is poorly understood. The Pou1f1dw(Pit1dw) mutant mice are deficient in pituitary thyrotropin (TSH), with no measurable thyroid hormone (TH), and exhibit profound deafness as assessed by auditory brainstem response (ABR). Although developmentally delayed, the morphology of the organ of Corti and expression of the outer hair cell motor protein prestin are nearly indistinguishable in six-week old mutants and normal littermates. The expression of two potassium channel proteins, KCNQ4 and KCNJ10, is permanently reduced in mutant cochlea, which may explain the absence of otoacoustic emissions and reduction of the endocochlear potential in these mutants. In addition, abnormalities in hair cell innervations are apparent in Pou1f1dw mutants, which could be the major contributor to the profound deafness observed in this hypothyroid strain. Genetic background affects the risk of hearing impairment in hypothyroid mice. To determine the complexity of the protective effects, an F1xF1 intercross was generated between Pou1f1dw carriers and an inbred strain of *Mus castaneus*. Approximately 25% of the mutant progeny exhibited ABR thresholds indicative of good hearing. A genome scan of these individuals revealed that a locus on Chromosome 2 can rescue hearing despite hypothyroidism. Microarray analysis identified cochlear gene expression changes caused by hypothyroidism in Pou1f1dw mice. Some of these are positional candidates for the modifier genes. We expect these studies to enhance our understanding of the mechanisms of hypothyroidism-induced hearing impairment