

P150**NOVEL C-KIT MUTATION KASUMI (KA) CAUSING STEM-CELL DEFICIENCY AND MODIFYING TESTICULAR AND OVARIAN TERATOCARCINOGENESIS IN KA-CONGENIC MICE**

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About 1 % of 129/Sv-Ter/(+/+) male mice and almost all of LTXBJ female mice develop spontaneously testicular teratomas and ovarian teratomas, respectively. We found a new coat-color mutation Kasumi (Ka) in a [129/Sv-Ter/(+/+) x LTXBJ] F1 female. Here we report the identification of the mutation by establishing 129/Sv-Ka/+ and LTXBJ-Ka/+ congenic strains. Ka mutation showed phenotypic effects similar to W (c-Kit on Chr.5) and SIJ (c-Kitl on Chr.10) mutations, including white coat with black eyes, anemia and sterility based on deficiency in pigment cells, erythrocytes and primordial germ cells. Evidence from mating tests between Ka/+ mice and W/+, Wv/+ or SIJ/+ mice and sequence analysis demonstrated clearly the occurrence of a novel miss-sense transition in exon 17 of the c-Kit gene in the Ka mutant gene. It was also indicated that the Ka mutation had occurred in the c-Kit gene from the strain 129/Sv-Ter/(+/+) not LTXBJ. Strikingly, for examples, 30 % of 129/Sv (Ka/+) males, 40 % of 129/Sv (Ka/+, SIJ/+) males, 80 % of 129/Sv (Ka/+, Ter/+) males had spontaneous testicular teratomas, whereas the Ka/+ males with the LTXBJ genetic background did not. It is first reported that a c-Kit mutation influences the incidence of testicular teratomas. About 50 % of the LTXBJ-Ka/+ females had ovarian teratomas. Thus, it is concluded that the Ka mutation is novel c-Kit mutation causing stem-cell deficiency in melanogenesis, erythrocytogenesis and gametogenesis and stimulating teratocarcinogenesis in primordial germ cells in the testes with 129/Sv strain background genes.

P151**SYSTEMS GENETICS APPROACH TO GENE-ENVIRONMENT INTERACTIONS**

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The immune system response to radiation illustrates the difficulty in defining health effects at low radiation doses. While high doses unequivocally suppress immune function, low doses may actually be immunostimulatory. Further, genetic background plays an important role in defining radiosensitivity. We are using systems genetics to dissect the polymorphism-transcriptome-physiological endpoint networks through which low dose radiation exposure impacts immune function, due to the immune system's hallmark sensitivity to radiation. This work exploits the availability of BXD (C57BL/6J X DBA/2J) recombinant inbred mouse strains and of a suite of analytical resources resident within WebQTL (genenetwork.org). A panel of physiological responses - inflammatory cytokines, oxidative stress response, immune function - are measured in parallel with transcriptomic responses in spleen across a panel of 40 BXD strains exposed to a single dose of 10 cGy of g-rays. Our goals are to 1.) define molecular mechanisms and pathways that underlie immune network responses to low dose radiation; 2.) test the hypothesis that genetic variability in those networks determines the immune outcome of low dose exposure; and 3.) produce an initial mapping of genetic regions that mediate the low dose response. Our long-term hypothesis is that some of the genetic factors that modify the radiation response also contribute to susceptibility to other conditions driven by oxidative stress and/or altered immune function (e.g., arthritis, cardiovascular disease). Use of a reference population will facilitate answering this question as data accumulate across time and space. Current results will be reported.

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P152**MULTI-DIMENSIONAL BEHAVIORAL, ALCOHOL AND DRUG ADDICTION PHENOTYPE ANALYSIS IN BXD RI MICE**

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The availability of integrative genomic resources for recombinant inbred lines, along with copious molecular phenotyping work available at GeneNetwork.org, and the completed expansion of the BXD strain set are a significant resource for the exploration of the genetics of drug and alcohol abuse susceptibility. These phenotypes are the consequence of multiple genetic predispositions including those which determine sensitivities to environmental events. Constructing gene-phenotype networks for alcohol and drug addiction requires behavioral profiling of the full panel of BXD RI lines to complement the increasing wealth of molecular data. Our ongoing phenotyping effort samples over 40 traits related to anxiety, depression, sleep, pain sensitivity, activity, novelty seeking, drug preference, sensitivity and response. Further, neurological phenotypes such as neurogenesis, and physiological phenotypes are also being obtained in this collaborative effort.

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P153**EXPLORATION OF COMPLEX EPISTATIC GENETIC CONTROL OF NASAL BONE SHAPE IN THE MOUSE USING INTERSPECIFIC RECOMBINANT CONGENIC STRAINS.**

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Genetic determinism of cranial morphology is complex and largely unknown in humans. Animal models such as mice may be useful in identifying genes influencing skull morphogenesis. In particular, inbred strains of several mouse species show substantial morphological differences.

We investigated skull shape variations between C57BL/6 and the *Mus spretus* derived SEG/Pas inbred strain. We concentrated on the shape of nasal bone, an almost flat structure easily amenable to description by outline analysis. To identify genetic loci accounting for these differences, we took advantage of a set of Interspecific Recombinant Congenic Strains (IRCS) resulting from crosses between C57BL/6 and SEG/Pas. Each of the 55 IRCS carries up to seven SEG/Pas chromosomal segments with an average size of 13.4 Mb, totalizing 1.26% of the genome. The complete series covers 38% of the SEG/Pas genome.

Nasal bone shape was studied in fifteen IRCS and compared with C57BL/6 using outline analysis with elliptic Fourier descriptors combined with canonical variate analysis. IRCS BcG-66H significantly differed from C57BL/6. An F2 cross between 66H and C57BL/6 revealed the presence of two QTLs, on Chr 1 and 18, with mostly additive effects. Each chromosomal region was then isolated in congenic strains to study single gene effects and combined in bi-congenic strains to analyze genetic interactions. Our results show that, besides a strong effect, QTL on chromosome 1 interacts with genes on chromosomes 13 and 18. This study demonstrates that genetic control of bone shape can be efficiently dissected in the mouse using appropriate tools.

P154**INTERSPECIFIC RECOMBINANT CONGENIC STRAINS BETWEEN C57BL/6 AND MICE OF THE MUS SPRETUS SPECIES : A POWERFUL TOOL TO DISSECT GENETIC CONTROL OF COMPLEX TRAITS**

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Complex traits are under the genetic control of multiple genes, often with weak effects and strong epistatic interactions. We developed two collections of mouse strains to improve genetic dissection of complex traits. They are derived from several backcrosses of the *Mus spretus* SEG/Pas or STF/Pas strains on the C57BL/6J background. Each of the 55 Interspecific Recombinant Congenic Strains (IRCS) carries up to seven SEG/Pas chromosomal segments with an average size of 13.4 Mb, totalizing 1.26% of the genome. This contribution of SEG/Pas is surprisingly low, seven times less than expected strains genealogy, and likely results from strong selection against *Mus spretus* alleles along inbreeding generations. The complete series covers 38% of the SEG/Pas genome. As a complementary resource, six partial or complete interspecific consomic strains (ICS) were developed, and increase genome coverage to 43.3%.

We are currently using the IRCS/ICS resource to seek genes controlling susceptibility to infectious diseases. Considerable differences are observed between mouse laboratory inbred strains and wild-derived strains. For example, after subcutaneous injection of 100 *Yersinia pestis* bacteria, the Plague agent, 90-100% C57BL/6 mice die within 5-6 days, while 85-100% SEG/Pas mice survive. Most IRCS did not differ from C57BL/6 in their susceptibility to Plague. However, several strains showed partial resistance, while a few others were killed more rapidly than C57BL/6. The last observation may reflect the existence of transgressive alleles, i.e. susceptibility alleles inherited from the resistant SEG/Pas strain. Altogether, this on-going study illustrates the usefulness of IRCS to study traits under complex genetic control.

P155**PHENOTYPIC ANALYSIS OF A SET DOMAIN-TRUNCATED MUTATION OF MOUSE ASH1**

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trithorax group (trxG) and Polycomb group (PcG) proteins were identified for their role in maintaining the transcriptional states of Hox and other developmental regulators, providing an epigenetic mechanism of cellular memory. In general, PcG proteins act as repressors to maintain the silent state, while trxG proteins are activators that maintain Hox gene transcription. In mammal, Hox genes are known to be involved in the vertebral axis formation and hematopoiesis. The absent, small or homeotic discs 1 (ash1) gene is a member of trxG. In *Drosophila*, loss of function mutations in ash1 causes homeotic transformations. Ash1 possesses a highly conserved SET domain which mediates histone lysine methylation. Recently it is reported that recombinant SET domain of mouse Ash1 can mono- and di-methylate histone H3 lysine 36 (H3K36), and that, in hematopoiesis, H3K36 methylation is required for activating Hox-A gene transcription and enforcing myeloid progenitor self-renewal. To clear the in vivo function of mouse Ash1 and H3K36 methylation, we have generated mice in which SET domain of Ash1 was deleted by homologous recombination (Δ SET). Heterozygous and homozygous Δ SET mutant mice were viable and fertile. They exhibited bidirectional homeotic transformations of axial skeleton. RT-PCR analysis using E11.5 Δ SET mutant embryos revealed no obvious change in the expression level of Hox genes. These results indicate that SET domain of Ash1 is required not for initiation but for fine-tuning of Hox gene expression to regulate vertebrate axis formation. Furthermore, we examined the Hox gene expression profile of hematopoietic cells from Δ SET mutant mice.

P156

REVERSIBLE CONTROL OF METASTASIS BY A PATHOGENIC mtDNA MUTATION

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It has been controversial whether mtDNA mutations are responsible for oncogenic transformation (normal cells to develop tumors), and for malignant progression (tumor cells to develop metastatic potential). It is possible to resolve this issue by isolating trans-mitochondrial cytoplasmic hybrids (cybrids) with mtDNA completely exchanged between cells that express different tumor phenotypes. Here we show convincing evidence that a missense G13997A mutation in the *ND6* gene of mouse mtDNA induces complex I defects and overproduction of reactive oxygen species (ROS), and reversibly controls the development of metastatic potential, but does not control the development of tumors. The development of metastatic potential as a result of the mtDNA mutation is not mediated by ROS-induced acceleration of genetic instability but rather by ROS-induced reversible upregulation of nuclear-coded genes related to apoptosis and neoangiogenesis. Thus, our findings of mtDNA-mediated reversible control of metastasis reveal a novel function of mtDNA, and suggest that ROS scavengers may be therapeutically effective in suppressing mtDNA-mediated metastasis.

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CANCER GENETIC STUDY FROM MOUSE TO HUMAN: CANCER RISK ASSOCIATED POLYMORPHISMS IN THE AURORA-A GENE

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Studies of mouse models of human cancer have shown the existence of multiple tumor susceptible genes that influence parameters of cancer such as transformation of cell, tumor multiplicity, tumor size, or the probability of malignant progression. Genetic susceptibility to common sporadic cancer is caused by abrogation of multiple genetic loci of low-penetrance genes as well high-penetrance genes. It is very important to reveal all tumor susceptible genes for early detection of cancer and screening of individuals who have higher cancer risk. We have analyzed skin tumor susceptibility in inter-specific (*Mus musculus* x *Mus spretus*) backcross mice treated with 7.12-dimethylbenz(a) anthracene (DMBA)/ 12-Otetradecanoylphorbol-13-acetate (TPA). We identified the gene encoding aurora kinase A (*stk6* in mouse and *AURKA* in human) as a candidate skin tumor susceptibility gene from the linkage analysis and haplotype mapping in the two-stage skin carcinogenesis model mice. Aurora kinase A (*AURKA*) gene product is a serine/threonine kinase essential for chromosome segregation and cytokinesis. There are two coding single nucleotide polymorphisms (cSNP), *AURKA*:c.91T>A and c.169G>A which create four haplotypes, 91T-169G, 91A-169G, 91T-169A, and 91A-169A. We evaluated the cancer risk in the haplotypes by case-control study in Japanese population and found 91T-169A and 91A-169A as higher cancer risk type. We observed significantly higher genome instability in esophageal cancer cells from patients with higher risk haplotype combination. Clinical follow-up data of esophageal cancer patients and study on molecular function of the aurora kinase A isoforms will be shared in this meeting for further understanding how *AURKA* polymorphism affect cancer risk.

P158**ANALYSIS OF CALCIUM OSCILLATION OF OOCYTES BY ARTIFICIAL MUTANTS OF SPERM-SPECIFIC PHOSPHOLIPASE C zeta IN THE MOUSE**

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During fertilization in mammals, the repetitive rise of intracellular Ca²⁺ concentration in oocyte, termed Ca²⁺ oscillation, is triggered by the penetration of sperm. In the mouse, the sperm-specific phospholipase C zeta (PLCzeta) is identified and has an ability to stimulate the Ca²⁺ oscillation in the oocytes. Therefore, PLCzeta is considered to be a powerful candidate of sperm factor. In the present study, the regulation of Ca²⁺ oscillation in mouse oocytes was investigated by the introduction of site-directed mutagenesis into the PLCzeta. Ca²⁺ oscillation in mouse oocytes injected with PLCzeta cRNA continued for 4 to 5 h and stopped at the period when pronuclei were formed. At the time of termination of Ca²⁺ oscillation, PLCzeta just accumulated in pronuclei. On the other hand, Ca²⁺ oscillation in mouse oocytes injected with the nuclear localization signal (NLS) mutant of PLCzeta cRNA continued although pronuclei were formed. This evidence was also confirmed by the transfer of pronuclei from mouse oocytes injected with the artificial mutant of PLCzeta into the unfertilized oocytes. Ca²⁺ transients and oocyte activation were detected in the reconstructed oocytes produced by the transfer of pronuclei from oocytes injected with PLCzeta cRNA into unfertilized oocytes, but were not in those by the transfer of pronuclei from oocytes injected with an inactive form and the NLS mutant of PLCzeta cRNAs. These results demonstrated that the initiation and termination of Ca²⁺ oscillation in mouse oocytes during fertilization are regulated by the oocyte-activating potential and the pronuclear translocation of the sperm-specific PLCzeta, respectively.

P159**PROTEOMIC ANALYSIS OF EARLY DEVELOPMENT IN MICE**Ryo Yamashita¹, Koichiro Miike², Masashi Aoki¹, Yumiko Takegawa¹, Ken-ichi Yamamura¹¹Division of Developmental Genetics, IMEG, Kumamoto University, ²Department of Child Development Kumamoto University

All mammals start development by sperm-oocyte fusion that is called fertilization. Fertilization generally consists of four major events, (1) Contact and recognition between sperm and oocyte, (2) Regulation of sperm entry into the oocyte, (3) Fusion of the genetic material of sperm and oocyte and (4) Activation of oocyte metabolism to start development. Before fertilization, oocyte arrests in metaphase of second meiotic division. At this stage, transcription is stop and translation is reduced in oocyte. Fertilization by capacitated sperm triggers the completion of meiosis, followed by formation of haploid maternal and paternal pronuclei. Meiotic maturation triggers the degradation of maternal RNAs. In the mouse embryo, transcription activation from zygotic genome, known as zygotic genome activation (ZGA) already starts pronucleus stage. In recent years, gene expression of early development in mice was discovered by transcriptome analysis. Transcriptome analysis has become to be the most powerful approach for global gene expression profiling, but the result may not reflect the direct function in vivo, since most transcripts do not have functions. On the other hand, proteomics analysis was established in recent years and the result obtained designates the direct function. So, we performed a differential analysis using a two dimensional gel electrophoresis that is the only top-down analysis method of proteomics available at the present time. We searched for essential factors for early development in mice.

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CHROMOSOME SEGREGATION IN MOUSE MEIOSIS

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The objective is to gain a molecular understanding of the mechanisms that regulate sister chromatid cohesion and separation in mammalian meiosis. Meiosis consists of two consecutive rounds of chromosome segregation that give rise to haploid gametes, egg and sperm. The multisubunit cohesin complex holds together sister chromatids from their generation in S phase until the second meiotic division. The resolution of chiasmata in meiosis I requires proteolytic cleavage along chromosome arms of the cohesin subunit Rec8 by separase. A second wave of proteolysis appears to trigger sister chromatid separation in meiosis II, presumably by separase-dependent cleavage of any remaining Rec8. A related cohesin subunit Scc1 is not essential in yeast meiosis but its cleavage by separase triggers sister chromatid separation in yeast and animal mitosis. Since Scc1 also appears to be present in mouse spermatocytes, it has been suggested that sister chromatids might be held together by Rec8- and Scc1-containing cohesin complexes in mammalian meiosis. Does Scc1 cleavage promote sister chromatid separation in mouse meiosis II? Alternatively, is Rec8 cleavage sufficient to trigger anaphase chromosome movements in meiosis II? We are addressing these questions using a novel cohesin cleavage assay based on a combination of genetics, protein engineering and live-cell imaging technology.

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WHIRLIN COMPLEXES WITH p55 AT THE STEREOCILIA TIP DURING HAIR CELL DEVELOPMENT

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Hearing in mammals is dependent upon the proper development of actin-filled stereocilia at the hair cell surface in the inner ear. Whirlin, a PDZ protein, is expressed at stereocilia tips and by virtue of mutations in the whirlin gene is known to play a key role in stereocilia development. We show that whirlin interacts with the MAGUK protein p55. p55 is expressed in outer hair cells in long stereocilia that make up the stereocilia bundle as well as surrounding shorter stereocilia structures. p55 interacts with protein 4.1R in erythrocytes and we find that 4.1R is also expressed in stereocilia structures with an identical pattern to p55. Mutations in the whirlin gene (whirler) and in the myosin XVa gene (shaker2) affect stereocilia development and lead to early ablation of p55 and 4.1R labelling of stereocilia. The related MAGUK protein CASK is also expressed in stereocilia in both outer and inner hair cells where it is confined to the stereocilia bundle. CASK interacts with protein 4.1N in neuronal tissue and we find that 4.1N is expressed in stereocilia with an identical pattern to CASK. Unlike p55, CASK labelling shows little diminution of labelling in the whirler mutant and is unaffected in the shaker2 mutant. Similarly, expression of 4.1N in stereocilia is unaltered in whirler and shaker2 mutants. p55 and protein 4.1R form complexes critical for actin cytoskeletal assembly in erythrocytes and the interaction of whirlin with p55 indicates it plays a similar role in hair cell stereocilia.

P162**NEURAL CREST DEFICIT OCCURS IN DOWN SYNDROME MICE AND IS ASSOCIATED WITH SONIC HEDGEHOG MITOTIC RESPONSE DEFICIT**Randall J. Roper^{1,2}, Justin VanHorn¹, Colyn C. Cain², and Roger H. Reeves²¹Indiana University-Purdue University Indianapolis, Indianapolis, IN ²Johns Hopkins University School of Medicine

Craniofacial abnormalities are distinguishing features of Trisomy 21. Both structural features and secondary sequelae adversely affect individuals with Down syndrome (DS). Because tissues affected in DS, including craniofacial skeleton, have a neural crest (NC) component, it has been hypothesized that trisomy 21 causes a defect in NC, but no direct experimental evidence supported or refuted this hypothesis. Ts65Dn mice are trisomic for orthologs of about half of the genes found on human chromosome 21 and exhibit DS-like abnormalities of the skull including brachycephaly, hypomorphic midface and small dysmorphic mandible. Quantitative analysis using unbiased stereology revealed a paucity of NC and a reduction in the size of the 1st branchial arch (BA1), the mandibular precursor, at midgestation in Ts65Dn as compared to euploid embryos. Subsequent quantification suggested deficits in trisomic NC generation, migration, and/or proliferation. Neural tube explants and cell culture assays showed deficits in migration and mitosis of trisomic NC. Sonic hedgehog (Shh) is associated with cerebellar hypoplasia in DS and is a known factor in NC and mandibular development. Addition of Shh overcame the trisomic mitotic NC deficiency in culture. These results provide the first direct demonstration that trisomy affects NC, and implicate Shh as a “common denominator” in multiple DS phenotypes. Understanding the developmental, cellular and molecular basis for abnormal trisomic craniofacial structure provides prospective targets for therapeutic intervention to ameliorate craniofacial and other DS phenotypes.

P163**MITOCHONDRIAL BOTTLENECK DUE TO THE REDUCTION OF mtDNA CONTENT IN THE FEMALE GERMLINE DOES NOT OCCUR IN MICE**Hiroshi Shitara¹, Liqin Cao^{1,2}, Takuro Horii³, Yasumitsu Nagao³, Hiroshi Imai³, Kuniya Abe⁴, Takahiko Hara¹, Jun-Ichi Hayashi², Hiromichi Yonekawa¹¹The Tokyo Metropolitan Institute of Medical Science, ²Graduate School of Life and Environmental Sciences, University of Tsukuba, ³Graduate School of Agriculture, Kyoto University, ⁴BioResource Center (BRC), RIKEN Tsukuba Institute

Mitochondrial DNA (mtDNA) defects are associated with a wide spectrum of serious genetic diseases. It is therefore critical to understand the segregation of mtDNA variants through germ line to the subsequent generations in order to assess the recurrence risks for mtDNA diseases. A narrow bottleneck in the number of mtDNA molecules, which occurs during early oogenesis, has long been proposed to explain the rapid mtDNA gene shifts in mammals. Using quantitative real-time PCR method, we estimated the mtDNA copy number in single germ line cells from various developmental stages in the mouse. We also quantified mtDNA content in single somatic cells of mouse embryos. Primordial germ cells showed consistent and moderate mtDNA content across the stages, providing direct evidence that the bottleneck is not mediated by mtDNA remarkable decline. Primary oocytes demonstrated substantial mtDNA expansion during early oocyte maturation, suggesting that the preferential amplification of subpopulations of mtDNA molecules at this stage may account for the mtDNA genotypic shift. Some somatic cells possess very low mtDNA, indicating a narrow bottleneck in somatic lineages during early cell differentiation. This is the first attempt to systematically investigate mtDNA content in single germ line and somatic cells of mice. These results provide basis for generating mtDNA segregation models and advance the prospects for prenatal diagnosis of mtDNA diseases.

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CHANGE OF PATHOGENIC MUTANT mtDNA WITH AGE IN MITOCHONDRIAL DISEASE MODEL MICE (MITO-MICE)

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The change of proportions of mutant and wild-type mtDNAs are crucial in determining the severity of mitochondrial diseases. It has been generally considered that deletion-mutant mtDNA has replication advantages and accumulates with age. Here, we examined the tissue-by-tissue proportions of pathogenic mutant mtDNA with a 4696-bp deletion (DmtDNA) and wild-type mtDNA in mitochondrial disease model mice (mito-mice). Comparison of the proportions of DmtDNA in each tissue with age showed that the rate of accumulation of DmtDNA differed among tissues. The heart, skeletal muscles, kidney, liver, testis, and ovary showed increases in the proportion of DmtDNA with age, but the pancreas, spleen, brain, and blood showed only a slight or no increase in proportion. In contrast to the somatic tissues, however, the germ cells of female mito-mice and resultant offspring showed a drastic decrease in DmtDNA with maternal age. The decrease was so acute that some offspring showed complete disappearance of DmtDNA, even though their elder brothers and sisters had higher proportions of DmtDNA. This interesting inheritance manner of DmtDNA might reflect the existence of quality control mechanism in female germ cells, which are kept in dormant for a long time until ovulation.

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SCREENING OF METASTASIS RELATED NON-CODING RNA IN MOUSE CELL LINES

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Metastasis is a major cause of death in cancer patients. Genomic alterations that affect the capacity of cells to regulate proliferation, signaling and metastasis have been observed in cancer. Furthermore, recent reports indicate that non-coding RNA can also contribute to cancer by regulating gene expression. In human malignant melanoma, many cancer-specific chromosome aberrations are reported. Recent studies precisely mapped such lesions by the use of single nucleotide polymorphism array. We used the information to explore the miRNAs affected frequently in cancer cells. The database analyses identified the 39 potential cancer related miRNAs in the aberrant human chromosomal regions. We are trying to refer the candidate miRNAs to those of mouse chromosomes. Subsequent functional analyses are being done with B16, a mouse melanoma cell line, and its variants. The B16 has several sublines with stronger metastatic potentials with various extents. Among them, we selected 5 candidate miRNAs which do not have any known cancer-related protein coding genes nearby. We also discuss the evolutionary conservation of miRNA-like non-coding RNAs between human and mouse identified by the FANTOM projects. Furthermore, to find the major driver genes for metastatic phenotype of the B16 variants, we performed expression profiling analysis to search for genes whose expression correlates with the strength of the metastatic phenotype among the B16 variants.

P166**ABNORMAL ASSEMBLY OF KERATIN INTERMEDIATE FILAMENTS IN MOUSE MUTATIONS OF TYPE I INNER ROOT SHEATH KERATIN**

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Keratins, a family of intermediate filament (IF) proteins, are composed of four α -helical rod domains, 1A, 1B, 2A and 2B. Type I (acidic) and type II (basic) keratin proteins form obligate heterodimers by the α -helical rod domains. They are involved not only in the mechanical and structural functions, but also in cell growth and differentiation of epithelial tissue. Mutations of several type II inner root sheath (IRS) keratin, such as Caracul (Ca) and its alleles, were reported, whereas no mutation has been reported for the type I IRS keratin. Two classical mouse hair coat mutations, Rex (Re) and Rex wavy coat (Re-wc), and an N-ethyl-N-nitrosourea (ENU)-induced mutation, M100573, are linked to the type I IRS keratin genes of chromosome 11. Three mutations confer a similar phenotype to Ca mice, with curly hair and vibrissae. In this study, we demonstrated that Re, Re-wc and M100573 have genetic alterations in type I IRS keratin genes. Re and M100573 bear mutation in Krt25, and Re-wc a mutation in Krt27. These three mutations are located in the helix termination motif of the 2B α -helical rod domain of the type I IRS keratin protein. Immunohistological analysis revealed that the IRS of Re/+ mice have abnormal foam-like immunoreactivity with an antibody raised to type II IRS keratin K71. These results suggest that the helix termination motif is essential for the proper assembly of type I and II IRS keratin protein complexes and the formation of keratin IFs.

P167**WHOLE GENOME MAPPING OF HISTONE H3 LYS4 AND 27 TRIMETHYLATIONS REVEALS DISTINCT GENOMIC COMPARTMENTS IN HUMAN EMBRYONIC STEM CELLS**

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Embryonic stem (ES) cells possess the ability to self-renew and differentiate into every tissue type in the adult animal. To explore the impact of epigenetic regulation on the ES cell identity, we profile the genome-wide H3K4me3 and H3K27me3 modifications by chromatin immunoprecipitation coupled with pair-end ditag (ChIP-PET) sequencing strategy. From saturated sequencing over, 17,000 H3K4me3 loci and 10,000 H3K27me3 loci are identified in human ES cells respectively. Our results clearly show that H3K4me3 is a predominant epigenetic marker in ES genome occupying two third of well annotated human genes. Interestingly, a subset of these genes is also simultaneously co-modified by H3K27me3, which displays overlapping but distinct profiles. When associated with gene expression activity, H3K4me3 could be regarded as a strong indicator of actively transcribed genes whereas genes with both markers are poised to be activated in immediately late development stage. Through the patterns of histone modification and associated gene expression activities, the ES genome can be categorized into distinct compartments implicated in the unique property of ES cells. This global histone methylation map significantly advances our understanding of mammalian epigenomics and the results shed light on mechanisms of epigenetic regulation on human ES cells.

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MOUSE EMBRYOS LACKING SFRP1 AND SFRP2 EXHIBIT ABNORMALITIES OF MALE SEXUAL DEVELOPMENT

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Secreted frizzled-related proteins (SFRPs) are soluble antagonists of WNT signalling implicated in a variety of biological processes. There are five members of the *Sfrp* gene family in mice. Targeted gene ablation of *Sfrp1* or *Sfrp2* individually results in mice with few overt abnormalities. However, mice lacking both genes die late in gestation and exhibit defects in anteroposterior axis elongation and somitogenesis, suggesting these two genes function in a redundant fashion. We have previously described expression of *Sfrp* genes in the developing mouse reproductive organs and here we describe an analysis of sexual development in embryos lacking both *Sfrp1* and *Sfrp2*. We firstly used a *lacZ* knock-in to study expression of *Sfrp1* in the reproductive organs and reveal its presence in structures associated with positioning of the gonad. At 16.5 dpc the most prominent phenotypic abnormality is failure of normal testicular descent in mutant males. The mutant vas deferens is frequently much shorter and projects upwards into the abdominal cavity. The developing testes are also smaller than littermate controls, with fewer testis cords. We will describe studies with a variety of gene and protein markers of the developing reproductive organs aimed at investigating the cellular and molecular basis of these abnormalities in the mutant male embryo. We will also present data showing the phenotypes associated with ENU-induced point mutations in *Sfrp2*.

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X CHROMOSOME REACTIVATION INITIATES IN NASCENT PRIMORDIAL GERM CELLS IN MICE

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X chromosome inactivation is a mechanism to compensate gene dosage difference between XY males and XX females in mammals. During early embryogenesis, one of two X chromosomes in every female cell is inactivated, and the inactive X chromosome is stably inherited through cell divisions of somatic cells. Although precise timing is not given, the inactive X chromosome is known to be reactivated during germ cell development. It is generally believed that the dynamics of X chromosome activity is tightly correlated with major genomic reprogramming events occurring during mammalian development. Therefore, elucidation of the X reactivation kinetics is important for understanding the mechanism of X chromosome inactivation/reactivation processes and the epigenetic reprogramming processes as well. Here we investigated when X reactivation is initiated during development of female mouse germ cells. Contrary to the previous suggestions, X reactivation already begins in nascent primordial germ cells in female mice and proceeds gradually requiring a prolonged period. The activity status of the X chromosomes of germ cells appears to vary from cell-to-cell and from gene-to-gene during the reactivation processes. These results indicate that the X reactivation coincides with the formation of germ cells and suggest that this involves slow passive steps.

P170**DEVELOPMENTAL ABNORMALITIES OF SPERM IN PCD MUTANT MICE IS ACCOMPANIED BY INSUFFICIENT NUMBER OF SERTOLI CELLS IN THE TESTIS**

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In Purkinje cell degeneration (*pcd*), an autosomal recessive mutation, homozygous mutant males show abnormal sperm development in addition to neuronal death. In mutant mice, the shape and motility of spermatids show severe defects, resulting in male sterility. We have analyzed the morphological differences of testes between wildtype and mutant mice through hematoxylin/eosin (H&E) staining. The microscopic examination of the testis in the mutant showed morphological abnormalities as previously reported. The number of haploid germ cells was also decreased in mutants. We find that the number of sertoli cells was decreased in the mutant testes from the histological evaluation. To further examine the identified differences between wildtype and mutant mice, immunohistochemistry using the sertoli cell specific antibody, Sox 9, was performed. Sox 9 positive signals were significantly reduced in adult mutant mice, compared to that of wildtype. From the TUNEL assay, apoptotic cells were identified from various stages of germ cells and sertoli cells. The abnormal sperm development including apoptotic death in *pcd* mutant mice could be attributed to the abnormal germ cell supporting environment caused by the death of sertoli cells.

P171**IMMUNOHISTOCHEMICAL AND FUNCTIONAL ANALYSIS OF PROTEIN 4.1 IN THE INNER EAR HAIR CELL**

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Protein 4.1 (band 4.1 or 4.1R) was originally identified as an abundant protein of the human erythrocyte, in which it stabilizes the spectrin/actin cytoskeleton. Additionally, different members, 4.1N, 4.1G and 4.1B have been identified. Recently, we have reported that 4.1R forms a complex with MAGUK protein p55 and two deafness gene products, myosinXVa and whirlin. To identify the detail functions of the 4.1 family members in developing inner hair cell stereocilia, we explored their expression in the developing inner ear hair cells of wild type and some mutant mice with stereocilia defects such as *wi*, *sh2*, *js*, (Jackson shaker), *v* (waltzer), *sv* (Snell's waltzer). The 4.1 family members were also expressed in the inner ear hair cells and showed specific expression patterns. Namely, 4.1R is expressed at the apical hair cell surface in early postnatal stages. The signals of 4.1R can be seen in the stereocilia and kinocilia. 4.1N was specifically expressed in the stereocilia bundle. In the cell body of hair cells, a high level of 4.1B was localized to the basolateral area where these cells form synapse with the dendrites of afferent neurons, and 4.1G was expressed in the perinuclear region of hair cells and supporting cells in the inner ear. Moreover, we found that some mutants lead to an ablation or mislocalization of their 4.1 proteins. These lines of evidence suggest that the 4.1 family members play important roles in the development and maintenance of the inner ear hair cells.

P172**ANALYSIS OF DIFFERENTIALLY METHYLATED REGIONS AMONG VARIOUS TISSUES AND DURING SKIN CARCINOGENESIS IN C57BL/6J MICE**Hiroki Nagase^{1,2}, Srymoyee Ghosh², Kyoko Fujiwara², Fei Song², William A. Held², Eiko Kitamura¹, Jun Igarashi¹, and Makoto Kimura^{1,2}¹Nihon University, 30-1 Oyaguchi, Kami-cho, Itabashi-ku, Tokyo, Japan, ²Roswell Park Cancer Institute, Buffalo, New York, USA

DNA methylation is one of the most important mechanisms to understand epigenetic effect in normal and cancer development. In order to understand mechanisms of DNA methylation, we developed a methodology of the global DNA methylation search using Restriction Landmark Genomic Scanning. 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 in a highly quantitative manner. Virtual image RLGS software (Vi-RLGS) in conjunction with real RLGS will make it possible to develop a more complete map of genomic sites that are methylated or demethylated as a consequence of normal or abnormal development. Skin tumors obtained from C57Bl/6J (B6) mice treated with a modified two-stage skin carcinogenesis protocol [induced by 7.12-dimethylbenz(a)anthracene (DMBA)/ 12-O-tetradecanoylphorbol-13-acetate (TPA)] were analyzed to understand aberrant DNA methylations in the promotion and/or progression stages of the skin carcinogenesis. We also applied this method to identify tissue-specific differentially methylated region (T-DMR) in B6 mice. Mouse T-DMRs are analyzed for normal development of tissues at three time points, 15 days embryonic development, newborn and 12 weeks adult. Some of orthologous regions of mouse T-DMRs and aberrant methylation changes in mouse skin cancers are also identified as tissue-specific/cancer specific differentially methylated regions in humans. This presentation will summarize hundreds of differentially methylated regions in normal and cancer development in several B6 tissues and comparative analysis of T-DMRs and aberrant methylation in cancers between the mouse and human. Interestingly some but not all of DMRs are conserved between mice and humans, and frequent reversible events in DNA methylation during normal and cancer development have been seen in mouse genomic sites.

P173**FUNCTIONAL ANALYSIS OF microRNAs IN HUMAN LUNG CANCER CELL LINE**Yukari Takahashi¹, Takehiro Hashimoto², Shiro Fukuda², Emi Maeno¹, Carsten Daub², Yoshihide Hayashizaki^{1,2} and Jun Yasuda¹¹RIKEN FRS RNA, ²RIKEN GSC

Accumulation of somatic genetic alterations and subsequent clonal expansions leads the cells to a malignant neoplasia formation. Because the “central dogma” is an almost ultimate paradigm in the molecular biology, cancer scientists mainly concentrated their attention to the protein coding genes as susceptibility genes for cancer. Hence they may not have paid enough attention to the possibility that RNAs are culprits of malignant human disorders. Nowadays the involvement of miRNA in carcinogenesis becomes apparent. In human lung cancers, many cancer-specific chromosome aberrations are reported. Recent studies precisely mapped such lesions by the use of single nucleotide polymorphism array. We are now identifying carcinogenesis-related noncoding RNAs localized in the frequently affected chromosomal regions in lung cancers based on a comprehensive study of chromosomal mapping of deleted or amplified regions in lung cancer cell lines. As an initial attempt, we are focusing on the microRNAs and identified 12 potential cancer related miRNAs in 7 repeatedly affected chromosomal regions. In total, including some singleton cases, we identified 15 candidate cancer related miRNAs. Among them, we selected 5 candidate cancer-related miRNAs which do not have any known cancer-related protein coding genes nearby and start to analyze those functions in a human lung cancer cell line. We will also describe the identification of noncoding RNAs in those chromosomal regions. Many functionally unknown noncoding RNAs are identified in the chromosomal regions affected in lung cancer cells. We will also discuss the evolutionary conservation between mouse noncoding RNAs identified by the FANTOM projects and present the functional analyses of the miRNAs in human lung cancer cell lines.

P174**USE OF THE SMART-AMPLIFICATION PROCESS FOR RAPID DETECTION OF EGFR MUTATIONS IN LUNG CANCER**

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It has been reported that mutations in Epidermal Growth Factor Receptor (EGFR) in non-small cell lung cancer (NSCLC) are correlated to a positive response to anti-cancer treatment with gefitinib. Previous reports studying these mutations have been based mainly on diagnostic screening by DNA sequencing. However, sequencing is a time-consuming and complicated procedure, not suitable for routine clinical use. We have developed a rapid, simple and sensitive mutation detection system named SMart Amplification Process (SMAP) and applied it for analyzing EGFR gene mutations in clinical samples. By using SMAP, we can detect mutations within 30 minutes including sample preparation. To test our method, we examined 45 NSCLC patients for EGFR mutations using direct sequencing and SMAP. The outcome of the SMAP assay perfectly matched the sequencing results, except in one case where SMAP was able to identify a mutation that was not detected by sequencing. We also evaluated the sensitivity and specificity of SMAP in mutation detection for EGFR. In a serial dilution study, SMAP was able to find a mutation in a sample containing only 0.1% of the mutation in a mixture of wild type genomic DNA. We also could demonstrate amplification of mutated DNA with only 30 copies / reaction. The SMAP method offers higher sensitivity and specificity than alternative technologies, while eliminating the need for sequencing to identify mutations in the EGFR gene of NSCLC. It provides a robust and point-of-care accessible approach for a rapid identification of most patients likely to respond to gefitinib therapy.

P175**A NODAL-INDEPENDENT LEFT-RIGHT ASYMMETRIC GENE MAY PROVIDE A DIRECT READ OUT OF NODAL FLOW**

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Vertebrates show left-right asymmetry in organ positioning and morphology. While much is now understood about the establishment of asymmetry in mammals, it is clear from mutant phenotypes that gaps exist in this understanding. Using a microarray-based screen we have identified a new asymmetrically expressed gene. We detect strong expression in the left lateral plate mesoderm, spatio-temporally overlapping that of Nodal, yet mutant analysis demonstrates that it is independent of Nodal. A second, highly dynamic, asymmetric expression pattern is seen at the node between 7.5dpc and 8.5dpc. Expression starts as a ring around the node, yet retreats in a clockwise direction around the node over the space of a few hours. Initial experiments suggest that the node domain is directly responding to nodal flow.

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MAPPING OF ALLELIC IMBALLANCES IN MURINE OSTEOSARCOMA USING WHOLE GENOME MD SNP ARRAYS REVEALS A NEW POTENTIAL TUMOR-SUPPRESSOR-LOCUS ON MMU 13

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Murine Osteosarcoma can easily be induced by injection with the osteotropic Alpha-Emitter Thorium227. Using F1 hybrid mice from different inbred strains allows mapping of tumor-specific allelic losses and - imbalances by comparative genotyping of normal tissue DNA versus tumor DNA. In order to use this approach with whole-genome coverage, we applied Illumina mouse MD SNP Arrays (Golden Gate) on a set of roughly 80 radiation-induced osteosarcoma induced in BALBxB6 and BALBxCBA mice. Chromosomal regions with potential allelic losses were identified using the LOH-module (part of Illumina Bead Studio Software) that is based on discrete genotypes, and compared with an procedure developed by our self that uses a continuous LOH-probability and adaptive thresholding. We show, that by masking non-informative SNP markers, followed by calculation of moving average and adaptive thresholding it is possible, to detect new chromosomal loci with osteosarcoma specific allelic losses. As proof-of-principle, we could verify frequent alterations at the chromosome 4 locus (P15/P16/P19), that has been mapped in an earlier study using microsatellite markers. The most recurrent new LOH locus in the present study was found on chromosome 13.

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HUMAN FCR-LIKE B CELL-SPECIFIC RECEPTORS: LIGAND SEARCHING

Konstantin Baranov, Ludmila Mechetina, Olga Volkova, Nikolai Chikaev, Alexander Taranin, Alexander Najakshin

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The family of FcR-like (FCRL) cell surface receptors is recently identified group of ancient immunoregulatory molecules differentially expressed on lymphocytes. While the signaling properties of FCRLs have been partly determined, little is known about their ligands. In search for the FCRL-interacting proteins, we generated soluble chimeric variants of two human B cell-specific members of the family, FCRL1 and FCRL4. These receptors have been previously shown to regulate B cell receptor signaling in a positive and negative manner, respectively. The extracellular parts of FCRL1 and FCRL4 were fused with placental alkaline phosphatase (AP) and produced in a eukaryotic expression system. FCRL-APs were tested as probes in binding to the FCRL1- and FCRL4-specific monoclonal antibodies immobilized on nitrocellulose membrane. This binding was found to be specific, sensitive, and easily detected due to the AP activity of the chimeric proteins. Staining of human tonsil cryosections with FCRL1-AP showed its weak interaction with cells of germinal centers. In contrast, FCRL4-AP strongly and specifically bound to cells scattered throughout the lymphoepithelium. Staining of FACS-isolated tonsil cell populations demonstrated that FCRL4-AP-binding cells belong to the CD3+ subset. These results suggest that FCRL4 ligand(s) may be expressed by T cells and that this receptor may participate in T-B cell interaction.

P178**A MICROARRAY BASED APPROACH FOR THE IDENTIFICATION OF GENES INVOLVED IN RESISTANCE/SUSCEPTIBILITY TO *H. PARASUIS* INFECTION**

Jamie Wilkinson

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Glässer's disease is an acute and usually fatal infection of the pig caused by the bacterium *Haemophilus parasuis*. It is characterized by fibrino-purulent inflammation of the serous membranes of the body. *H. parasuis* is commonly isolated from the respiratory tract of healthy pigs where it lives as a commensal organism. Therefore a number of bacterial, host and environmental factors probably determine whether the bacteria breach the mucosal epithelial barrier to cause systemic disease.

In order to investigate the role that host genetic factors play in resistance/susceptibility to disease, a series of animal challenge experiments were carried out. Piglets that had been artificially reared in sterile conditions and deprived of maternal antibodies were infected with a pathogenic strain of the bacteria and euthanized at 24, 48, or 72 hours post-infection. The animals were scored for the presence of disease, and two "resistant" groups of animals were identified - a "resistant A" group that showed no signs of infection (confirmed by RT-PCR on lung samples for *H. parasuis* 16S rRNA gene) and a group of animals that exhibited reduced signs of systemic disease ("resistant B" group). Microarray experiments comparing gene expression in the lung and lymph node of "resistant" animals and matched susceptible animals are currently being carried out. Candidate resistance genes identified through the microarray experiments will then be screened for single nucleotide polymorphisms (SNPs). Finally, association studies will be carried out on a set of resistant and susceptible animals to determine whether particular SNPs are associated with resistance/susceptibility to Glasser's disease.

P179**MICROBIAL DIVERSITY IN INTESTINAL TRACTS OF TWO SPECIES OF RODENTS IN MONTANE AREAS IN TAIWAN**

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Microorganisms living in mammals' guts have important effects on immune function, nutrient processing and other host activities. We analyzed the bacterial communities in three sections of the digestive tracts, including small intestines, caeca and large intestines of two montane species of rodents (*Microtus kikuchii* and *Apodemus semotus*) from the central mountain range in Taiwan. We constructed 16S rDNA clone libraries and identified the sequences through database search. At the phylum level, the intestinal microflorae of *Microtus kikuchii* were composed of *Proteobacteria* (40.68%), *Firmicutes* (32.70%), *Bacteroidetes* (22.05%), *Spirochaetes* (1.90%), *Actinobacteria* (1.52%), TM7 (0.76%) and *Deferribacteres* (0.38%). The intestinal microflorae of *Apodemus semotus* were composed of *Firmicutes* (42.26%), *Proteobacteria* (42.23%), *Deinococcus-Thermus* (9.84%), *Verrucomicrobia* (2.33%), *Cyanobacteria* (1.81%) and *Actinobacteria* (0.52%). We also found that the bacterial composition percentages between three gut sections of the same individual differed from one another. Moreover, while the SPF mouse and human microflorae were dominated by *Firmicutes* and *Bacteroidetes*, respectively, the two species have a large group of the phylum *Proteobacteria* which comprises a huge number of environmental bacteria. This dissimilarity suggests that wild mice have specific immune tolerance for some of *Proteobacteria* and these microbes may own unique metabolic traits to colonize the gut. This research offers an opportunity to know the commensal host-bacterial relationships in the gut of wild rodents.

P180**ESTABLISHMENT OF AN EOSINOPHIL-LESS MOUSE LINE BY TRECK METHOD**Kunie Matsuoka¹, Asuka Motoda¹, Hiroshi Shitara¹, Kenji Kohno² and Hiromichi Yonekawa¹¹Laboratory of Mouse Models for Human Heritable Diseases, The Tokyo Metropolitan Institute of Medical Science (RINSHOKEN) and ²Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST)

Eosinophils are common dominant inflammatory cells which exist in the skins of atopic dermatitis and in the epidermis of airways of asthma patients. We have previously shown that massive eosinophil infiltration was observed in the chronic phase ear swelling in IgE-mediated allergic inflammation initiated by basophil (*Immunity* **23**, 191-202, 2005). Nonetheless, the role of these leukocytes in such allergic diseases remains poorly understood. To address this issue, we tried to establish a novel model mice that allows us the inducible *in vivo* ablation of eosinophils. The eosinophil-less mouse model (C57BL/6-EPO-TRECK-Tg mice) was generated by transgenesis of human DT receptor (HB-EGF) gene under the control of eosinophil peroxidase (EPO) promoter. Intraperitoneal administration of DT caused selective depletion of eosinophil in peripheral blood. Treatment of Tg mice with DT suppressed the delayed-onset allergic ear swelling elicited by a single intradermal injection of the antigen TNP-OVA in mice that had been passively sensitized with a TNP-specific IgE. The eosinophil-less mouse model should be a powerful tool for studying the *in vivo* roles of eosinophils, not only in allergic inflammation but also in immune regulation and protection from pathogens.

P181**CONDITIONAL GENE TARGETING OF CYP51 (LANOSTEROL 14a-DEMETHYLASE)**Helena Motaln¹, Natasa Debeljak², Damjana Rozman², Simon Horvat¹¹Univ. of Ljubljana, Biotechnical Faculty, Dept. of Anim. Science, Groblje 3, 1230 Domzale, Slovenia. ²Univ. of Ljubljana, Faculty of Medicine, Instit. of Biochemistry, Center for Function. Genomics and Bio-Chisps, Zaloska 4, 1000 Ljubljana, Slovenia

Lanosterol 14a-demethylase encoded by *Cyp51* is an important house keeping gene involved in cholesterol biosynthesis. Additionally, in the germ cells, the mammalian *Cyp51* produces meiosis-activating sterols (MAS), biologically active post-lanosterol intermediates of cholesterol biosynthesis. MAS reinitiate oocytes meiosis *in vitro* while *in vivo* they appear to contribute to the oocyte quality and meiosis progression. In the male germ cells, *Cyp51* expression and MAS production increase significantly during the round spermatozoid maturation into its elongated form implying a functional role of *Cyp51* and MAS during spermatogenesis. To test this implied functional role *in vivo*, we propose to generate the conditional *Cyp51* knockout mice model. The *Cyp51* gene inactivation would be restricted to specific cells at specific developmental periods enabling us to investigate the molecular basis of *Cyp51* involvement in the spermatozoid remodelling. We intend to utilize the Flp/frt recombination system for elimination of the marker gene from the ES cells' genome and testis specific CRE-expression mice for temporal regulation of *Cyp51* inactivation in mice. So far, four ES cell lines successfully targeted and treated with Flip -recombinase that excised the selection marker cassette were generated. Experiments with chimera and inducible *Cyp51* inactivation mouse model generation on the pure C57BL/6 genetic background are under way. This model could prove valuable in future not only for assessing the spermatogenesis phenotype proposed above but could also be of interest to the wider scientific community to allow conditional *Cyp51* inactivation in various tissues and developmental time points.

S1-5/P182**QUANTITATIVE TRAITS FOR THE TAIL SUSPENSION TEST: AUTOMATION, OPTIMISATION AND BXD RI MAPPING**

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Immobility in the tail-suspension test (TST) is considered a model of despair in a stressful situation, and acute treatment with antidepressants reduces immobility. Inbred strains of mouse exhibit widely differing baseline levels of immobility in the TST and several Quantitative Trait Loci (QTLs) have been nominated. The labour of manual scoring and various scoring criteria make obtaining robust data and comparisons across different laboratories problematic. Several studies have validated strain gauge and video analysis methods by comparison with manual scoring. We set out to find objective criteria for automated scoring parameters that maximise the biological information obtained, using a video tracking system on tapes of tail suspension tests of 24 lines of the BXD recombinant inbred panel and the progenitor strains C57BL/6J and DBA/2J. The maximum genetic effect size is captured using the highest time resolution and a low mobility threshold. Dissecting the trait further by comparing genetic association of multiple measures reveals good evidence for loci involved in immobility on chromosomes 4 and 15. These are best seen when using a high threshold for immobility, despite the overall better heritability at the lower threshold. A second trial of the test has greater duration of immobility and a completely different genetic profile. Frequency of mobility is also an independent phenotype, with a distal chromosome 1 locus.

S2-4/P183**MAPPING AND CHARACTERIZING ENU-INDUCED MUTANT MOUSE MODELS OF THROMBOCYTOPENIA**

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Thrombocytopenia is characterized by low levels of circulating platelets which can result in uncontrolled bleeding. Two thrombocytopenic mouse strains were obtained from the ENU mutagenesis program at The Jackson Laboratory's Heart, Lung, Blood and Sleep Disorders Center. We mapped the recessive HLB219 and HLB381 mutations using an F2 intercross strategy. Candidate gene sequencing of HLB219 revealed a point mutation in the *Mpl* gene. The MPL receptor and its ligand TPO, play a major role in megakaryocyte (Mk) proliferation, maturation, and platelet production. Bone marrow from HLB219 has a reduced number of hematopoietic progenitors and fails to effectively contribute to hematopoiesis in competitive repopulation assays. In contrast, BaF3 cells expressing *mpl*^{h219} proliferate independently of TPO. Furthermore, heterozygous mice have an increased platelet count despite reduced Mk levels. Thus the mutant protein may have a low level of constitutive activity related to at least one downstream signaling pathway.

The HLB381 mutation was mapped to a 4.2 megabase interval on Chr8 by a genome-wide linkage scan. In contrast to HLB219, HLB381 bone marrow demonstrates a normal hematopoietic potential in a competitive repopulation assay. Chimeric mice from these assays, however, have low platelet levels suggesting a defect late in platelet biogenesis or in platelet lifespan. HLB381 mice have normal CFU-Mks and normal levels of circulating TPO. Current efforts are focused on examining the rate of platelet turnover and early platelet biogenesis. The narrowed region has no known genes related to platelet biology and identification of the mutation will likely advance our understanding of thrombopoiesis.

P184**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

S1-4/P185**PHENOTYPIC EFFECTS OF THE “MINI-MUSCLE” ALLELE IN A LARGE HR x C57BL/6J MOUSE BACKCROSS**

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From outbred Hsd:ICR mice, we selectively bred four replicate lines for high running (HR lines) on wheels, while maintaining four non-selected lines as controls (C lines). An apparent Mendelian recessive, the “mini-muscle” (MM) allele, whose main phenotypic effect is to reduce hindlimb muscle mass by 50%, was discovered in two HR lines and one C line. This gene of major effect has gone to fixation in one selected line, remains polymorphic in another, and is now undetectable in the one control line. Homozygotes exhibit various pleiotropic effects, including a doubling of mass-specific muscle aerobic capacity, and larger hearts, livers, and spleens. To begin mapping the genomic location of the MM allele and to better characterize its pleiotropic effects, we crossed females fixed for the MM allele with male C57Bl/6J. F1 males were then backcrossed to the MM parent females. Backcross mice (N = 404) were dissected, and a 50:50 ratio of normal to MM phenotype was observed with no overlap in relative muscle mass. In the backcross, analysis of covariance revealed that MM individuals ran significantly more on days 5 and 6 of a 6-day exposure to running wheels, were smaller in body mass, and had larger ventricles and spleens.

S1-1/P186**UNFOLDING POPULATION STRUCTURE AND ANALYSING GENETIC VARIABILITY OF ZALAWADI, GOHILWADI AND SURTI GOAT BREEDS OF GUJARAT(INDIA) USING MICROSATELLITES**Shadma Fatima¹, C. D. Bhong², D.N. Rank³, C.G. Joshi⁴¹MVSc, Dept of Animal Genetics and Breeding, ²Research fellow, ³Assistant professor, ⁴Professor and Head

India is bestowed with 17% of total world goat population comprised of about 21 pure breeds and many non descripts. With 21 breeds in India, Gujarat is having some of the best goat breeds having high genetic potential viz. Zalawadi, Gohilwadi and Surti goat breeds. They are important genetic resources to be conserved and characterization of indigenous germplasm and analysing population structure is essential for their conservation. In the present study eighteen microsatellite pairs were chosen from the list suggested by ISAG and amplified in two multiplexes. The observed number of alleles ranged from four (Oar JMP-29) to fifteen (ILSTS-030 and -034) with a total of 178 alleles and mean of 9.89 alleles across three breeds. The overall heterozygosity, PIC and Shannon index values were 0.61, 0.60 and 1.50 indicating high gene diversity. The highest observed heterozygosity was found in Gohilwadi and minimum in Surti goat breed. Genetic distance was least (0.128) between Gohilwadi and Zalawadi and highest between Gohilwadi and Surti (0.1951). In all populations no inbreeding was indicated (mean $F_{IS} = 0.0192$, $F_{IT} = 0.0914$) within and among the breeds. Genetic differentiation between breeds was moderate with a mean F_{ST} value of 0.073 which showed that the average proportion of genetic variation explained by breed differences was 7.3%. A dendrogram using UPGMA clustering was generated from Nei's genetic distance, followed the geographic origin of the breed. The exclusion-simulation significance test assigned individuals with 96.2% accuracy (e.g., $P < 0.01$) when using first 14 microsatellites ranked on the basis of accuracy of assignments individually. This extensive research on goat genetic diversity provides valuable information, to understand the relative distinctiveness of goat genetic resources, and will assist in developing a plan for the conservation and utilization of indigenous goat breeds.

Key words: Microsatellite, Zalawadi goat, Gohilwadi goat, Surti goat, genetic diversity.

S2-6/P187**PSTPIP2 IS MUTATED IN THE FIREWALKER MOUSE TO CAUSE AN AUTOIMMUNE DISEASE**

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N-Ethyl-N-Nitrosourea (ENU) is a highly efficient mouse mutagen that induces random mutations to isolate virtually any component required for developmental and pathological processes. From this approach, a recessive mutant line with a scabby kink-tailed phenotype was isolated and named Firewalker. The visible symptoms appear as joint swelling in the digits or feet at about 1-3 months after birth. However, we found that macrophage cell expansion and erythrocyte reduction in the bone marrow precedes this outward manifestation of the phenotype. Histological analysis of Firewalker feet and ears reveals inflammatory invasion in the epidermis and dermis layers of the skin. Bone deformation is observed in the tail and tibia, and ankle joints are completely disorganized. Splenomegaly is a common feature, likely because it is compensating for blood formation, since the bone marrow is filled with macrophages. In addition, the stomach of Firewalker is deformed by inflammatory infiltration that may result in digestive trouble, and explains the progressive weight loss of mutants. From these results, Firewalker appears to be an autoimmune disease such as rheumatoid arthritis and in support of this contention, we detect anti-nuclear antibody in the serum of mutants. We mapped Firewalker to a 4 Mbps region of chromosome 18 by microsatellite marker analysis, and found a mutation in the proline-serine-threonine phosphatase interacting protein 2 (*Pstpip2*) gene. *Pstpip2* was first isolated as a macrophage actin-associated protein, which regulates F-actin bundling and enhances filopodia formation and motility in macrophages, making it an excellent candidate for this autoimmune disease.

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GENETIC DISSECTION OF MOUSE ANXIETY-RELATED BEHAVIOR

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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.

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GENETIC POLYMORPHISM STUDY OF IGF-I GENE IN DAIRY BUFFALOES OF GUJARAT (INDIA).

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The IGF plays an important role in lactation. Polymorphs of IGF gene are reported to be significantly associated with milk production and constituent traits. A study was undertaken to detect polymorphism at the genetic level and to explore allelic variability at this locus. A total of 150 animals, belonging to three breeds of riverine buffalo viz. Mehsani, Surti and Jafarabadi were scanned. The gene was amplified by polymerase chain reaction revealing a product of 265bp and subsequently, single strand confirmation polymorphism (SSCP) study was carried out to identify different allelic pattern and genotypes of the animals. All the three breeds exhibited polymorphism yielding three different SSCP patterns, the sequence analysis could not confirm polymorphisms. However, three polymorphic positions were detected in the complete genomic IGF exon I region of these buffalo breeds than the reported one. The sequence showed variation at three positions (at 89C/T, 98G/T and 167T/C) when compared with reference sequence. Information provided in this study will be useful in further studies to determine the role of IGF in the regulation of milk synthesis and improvement of quality and quantity for milk in riverine buffaloes.

Key Words: IGF-I gene, Mehsani buffalo, Surti buffalo, Jafarabadi buffalo, SSCP, Sequencing

P190**AN N-ETHYL-N-NITROSOUREA MUTAGENESIS SCREEN IN MOUSE IDENTIFIES A CANDIDATE REGION FOR CARDIOMYOPATHY IN THE PROXIMAL END OF CHROMOSOME 1**

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N-ethyl-N-nitrosourea (ENU) mutagenesis in the mouse is a powerful tool to create novel mutations. Using this approach, mutations are generated at random across the genome and offspring are screened for phenotypes of interest. We have performed an ENU mutagenesis recessive screen in adult mice to identify novel disease-causing and disease-modifying genes for cardiomyopathy. Using non-invasive echocardiography to screen for abnormalities in cardiac function, we have identified a family (EN1) with heritable cardiomyopathy. To identify the chromosomal region where the mutation is localized, we used whole genome single nucleotide polymorphism (SNP) genotyping. By this method, we identified the mutagenized region at the proximal end of chromosome 1. Family EN1 has 66 mutants and by using microsatellite markers and additional SNPs, we have narrowed the candidate region to an interval of ~2 Mb. There are 18 genes in this interval such as Nur77 downstream gene 1, Transmembrane protein 14a, and Potassium voltage-gated channel, subfamily Q, member 5. Interestingly, there are no sarcomeric protein genes within this interval. Gene expression analysis and sequencing of the candidate genes are ongoing to identify the gene. A second family (EN25) with 8 mutants has also been identified. The mutagenized region maps to the distal end of chromosome 15 and we have narrowed it to an interval of ~12 Mb. Our recessive ENU mutagenesis screen has allowed us to map two chromosomal regions associated with dilated cardiomyopathy. The identified genes in this screen will be strong candidates for disease-causing and disease-modifying genes in patients with heart failure.

P191**GEMIN2 PLAYS AN IMPORTANT ROLE IN STABILIZING THE SURVIVAL OF MOTOR NEURON COMPLEX**

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The survival of motor neuron (SMN) protein, responsible for the neurodegenerative disease spinal muscular atrophy (SMA), oligomerizes and forms a stable complex with seven other major components, the Gemin proteins. Besides the SMN protein, Gemin2 is a core protein that is essential for the formation of the SMN complex, although the molecular basis of the mechanism behind the formation has not been clarified. We have found a novel interaction, a Gemin2 self-association, using the mammalian two-hybrid system and the *in vitro*/ pull-down assays. Using *in vitro* dissociation assays, we also found that the self-interaction of the amino terminal SMN protein, which was confirmed in this study, became stable in the presence of Gemin2. In addition, Gemin2 knockdown using siRNA treatment revealed a drastic decrease in SMN oligomer formation and in the assembly activity of spliceosomal small nuclear ribonucleoprotein (snRNP). Taken together, these results indicate that Gemin2 plays an important role in snRNP assembly through the stabilization of the SMN oligomer/complex via the novel self-interaction. Applying the results/techniques to the amino terminal SMN missense mutants that have been recently identified from SMA patients, we could successfully show that amino terminal self-association, Gemin2 binding, the stabilization effect of Gemin2 and snRNP assembly activity were all lowered in the mutant SMN(D44V), suggesting that instability of the amino terminal SMN self-association may cause SMA in patients carrying this allele.

P192**HAIR-LOSS MUTATION (DEP) CAUSED BY A MUTATION IN PALMITOYL TRANSFERASE ZDHHC21**

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Palmitoylation is a post-translational modification that involves the addition of the fatty acid palmitate onto specific cysteine residues. Recently, several members of a family of transmembrane proteins containing a zinc finger and a DHHC motif, have been shown to be palmitoyl transferases in yeast and mammalian cells.

The recessive hair loss mutant, *dep*, contains a mutation (del-233F) at the C-terminal of *Zdhhc21*. Wild-type *Zdhhc21* has been shown to enhance palmitoylation of several specific substrates in a transfected cell assay. *Zdhhc21* localises to the cis-Golgi, whereas the mutant protein is mislocalised and is inactive in palmitoylation. We verified the candidacy of *Zdhhc21* by transgenic BAC rescue.

Dep is characterised by progressive hair loss, hyperplasia of the sebaceous glands, the interfollicular epidermis and the outer root sheath. *In-situ* hybridisation and immunohistochemistry show that both wild-type and *dep* mRNA and protein are present in the inner root sheath (IRS).

Phenotypic characterisation using molecular markers in cell culture and on skin sections reveals abnormalities that suggest a lack of correct hair shaft differentiation in *dep*. We speculate that *dep* may have a direct or indirect effect on 4 members of the Wnt family - essential regulators of hair shaft differentiation - because of their co-expression in the IRS and because *dep* exhibits a Wnt-deficient phenotype.

This hypothesis may provide an example of how local signalling centres may be established to allow for spatiotemporal gene expression. Furthermore, *dep* is the first mouse model that provides direct evidence of an enzymatic activity of the Dhhc family.

P193**TRANSCRIPTION REGULATORY CASCADES IN RETINOIC ACID-INDUCED GROWTH ARREST OF HEPG2 CELLS.**

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All-trans retinoic acid (ATRA) treatment of mammalian cells induce expression of a variety of genes and are influenced on their biological processes, especially cell proliferation and differentiation. In HepG2 cells, human hepatocellular carcinoma cells, ATRA induced growth arrest within 48hrs after treatment. We found 719 genes which changed expression level more 2-fold than non-ATRA treatment cells at five time points after treatment. Then, we focused cell-cycle related 54 genes and searched transcription regulatory factors (TRFs) which bind to their promoter regions, from -2000bp to +200bp relative to the representative transcription start sites, with TRANSFAC MATCH program. Because the expression changes of regulated genes occur later than its of TRF genes, we select 61 TRF genes which expression changed before the expression of candidates of regulated genes changed. In these TRF genes, we chose six TRF genes (CEBPA, DDIT3, EGR1, RARA, RARB, SREBF1) as targets to identify their regulated genes by knockdown and chromatin immune precipitation of them. Here, we identified the transcription regulatory cascades which start from six TRF genes in the process of anti-proliferation effect of ATRA in HepG2.

P194**IDENTIFICATION OF CONSERVED DNA REGIONS AND A SET OF TRANSCRIPTION FACTORS INVOLVED IN COMBINATORIAL REGULATION OF SEVERAL HUMAN LIVER-ENRICHED TRANSCRIPTION FACTOR GENES**

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Tissue- and/or time-specific transcription is primarily regulated in a combinatorial fashion through the interactions between a specific set of transcriptional regulatory factors (TRFs) and their cognate cis-regulatory elements located in the regulatory regions including a proximal promoter. Identification of a set of TRFs and detection of DNA regions involved in the combinatorial regulation of the gene in question is essential for understanding the mechanism of its transcriptional regulation. Recent genome-wide location analyses detected TRF-binding sites in a variety of locations such as 5'- and 3'-untranslated, coding, intronic, and even intergenic regions, raising a question which sites are involved in active transcriptional regulation. Here, we explored a method for identification of the DNA regions and TRFs involved in a combinatorial transcriptional regulation (CTR). We then searched the potential binding sites for five TRFs (TCF1, FOXA1, FOXA2, FOXA3, and HNF4A) in the conserved genomic regions around human FOXA3, TCF1, and CEBPA genes whose expressions were perturbed by RNAi knockdown of these TRFs. Chromatin immunoprecipitation (ChIP) analysis revealed that almost all of these DNA regions were bound by these five TRFs as well as two coactivators (CBP and P300), strongly support that these DNA regions and TRFs may be involved in CTR. We also found a clear preference of the DNA regions containing multiple TRF binding sites in fragment enrichment by ChIP to those containing a single TRF binding site, suggesting the preferential TRF binding to the specific sites in combinatorial regulatory regions over single TRF binding sites.

P195**GENOME-WIDE ANALYSIS ON ABNORMAL H3K9 ACETYLATION IN ADULT CLONED MICE**

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Somatic nuclear transfer cloning is a promising to apply to therapeutic applications. Although Cloned animals are expected to be identity to their donors, several abnormal phenotypes, such as obesity and abnormal gene expression are observed. The one of the causes of the abnormalities is hypothesized as an incomplete reprogramming on epigenetic memories of donor cells. However, relationship between epigenetics and phenotypes is not still clear. Here we genome-widely demonstrate abnormal H3K9 acetylation (H3K9Ac) in cloned mice, identifying a notable difference on the H3K9Ac of certain genes including Crp. We also provide evidence showing that the abnormal H3K9Ac causes abnormal gene expression. Furthermore, we demonstrate that the level of the CRP protein in the blood shows strong positive correlation to weight, indicating that the Crp is related to obesity of cloned mice. We suggest that the abnormal H3K9Ac is one of the origins for the abnormal phenotypes in cloned mice.