

P50**MULTIPLEX CDNA QUANTIFICATION METHOD BY DNA ENCODING TECHNOLOGY**Osamu Gotoh¹, Atsushi Kameda¹, Yasufumi Murakami², Akira Suyama¹¹The University of Tokyo, ²Tokyo University of Science

Gene expression analysis is increasingly important for biological and medical research. Currently major gene expression profiling methods are based on DNA microarrays and quantitative PCR (qPCR). A DNA microarray-based method is highly parallel, so that thousands of genes can be analyzed all at once. However, the method is not so quantitative that it can only determine relative changes in gene expression levels. In contrast, a qPCR-based method is known as most sensitive and quantitative. However, many standard templates to generate a standard curve are needed to determine the absolute amount of cDNA. Therefore it is time-consuming to measure a bunch of cDNAs. Here, we report a cDNA quantification method (DEAN: DNA Encoding-based ANalysis) for gene expression profiling with the advantages of both a DNA microarray and a qPCR-based method. This approach is based on DNA encoding technology of gene expression levels to DNA-Coded Numbers (DCNs) that are special DNA-tag sequences. The validity of this method was confirmed by using the test samples composed of synthetic DNA strands of known quantities. It was found to be highly sensitive and more accurate than qPCR. Further validation using cDNA samples prepared from mouse liver revealed the accurate quantification of actual cDNAs. These results also demonstrated 294-multiplex quantifications with the sensitivity of 0.01 attmoles (10^{-20} mol), that are corresponding to total RNA 0.1 micro g. Therefore DEAN will give a promising approach to cDNA quantification for standardized gene expression profiling in transcriptomics and clinical diagnostics.

P51**CAGE-TSSCHIP; PROMOTER-BASED EXPRESSION PROFILING USING THE 5'-LEADING LABEL OF CAPPED TRANSCRIPTS**Shintaro Katayama¹, Mutsumi Kanamori-Katayama¹, Yuki Tsujimura¹, Noriko Ninomiya¹, Kazumi Yamaguchi^{1,2}, Piero Carninci^{1,3}, Yoshihide Hayashizaki^{1,3}¹Genome exploration research group, genomic sciences center, RIKEN. ²Nittetsu Hitachi Systems Engineering, Inc. ³Genome science laboratory, RIKEN.

The Cap Analysis Gene Expression (CAGE) technology has revealed numerous transcription start sites (TSSs) in mammals. Promoter identification and promoter-based expression profiling are essential to understand the regulatory networks. However, traditional technologies for expression profiling did not represent the activity of each TSSs, and deep sequencing is required if we use CAGE or similar technologies. Therefore, an alternative technology to large-scale promoter-based expression profiling was required. We developed the CAGE-TSSchip to detect the promoter-based transcriptional activity. It is a customized oligo-array that targets known TSSs identified by CAGE, and our new labeling method must be applied. This new method labels capped transcripts from the 5'-end. We performed two direct comparisons of two samples using CAGE-TSSchip, and validated these results using the other methods. First, the CAGE-TSSchip showed the similar expression ratio as the quantitative RT-PCR (qRT-PCR) and the CAGE technology, and the results correlated well even for rare transcripts. The expression (intensity) ratio for 5'-side probes tended to be markedly different than the 3'-side probes. Moreover, when making a comparison between Hepa1-6 and liver, the CAGE-TSSchip extracted the promoters of many proliferation-related genes and suggested that one of the appropriate key regulators of these promoters is the E2F transcription factor 1. The CAGE-TSSchip has accuracy and sensitivity, and represents the activity of each TSS. It is clear that promoter-based expression profiling is very important to draw a network of transcriptional regulatory pathways, and that this CAGE-TSSchip is a useful tool for the promoter-by-promoter network analysis.

P52**GENETIC CONTROL OF GENE EXPRESSION AND GENE NETWORK IN THE HIPPOCAMPUS OF LXS MICE**Lu Lu¹, Melloni Cook², Beth Bennett³, Robert Williams¹¹Department of Anatomy and Neurobiology, University of Tennessee Health Science Center, Memphis, TN 38163;²Department of Psychology, University of Memphis, Memphis, TN 38152; ³Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309

In addition to roles in memory and learning, the hippocampus is a key component of vulnerable system that regulates responses to stressors. Over the past 30 years neurogeneticists have accumulated a wealth of data on genetic differences in anatomical and functional characteristics of mouse hippocampus. This variation is partly generated by DNA sequence variants that control mRNA expression level. We have used the Illumina array to quantify mRNA expression in adult hippocampus of 77 LXS strains made by crossing Inbred Long and Short Sleep (ILS and ISS) strains, selected for their marked differences in response to ethanol. Expression data were used to map QTLs associated with variation in expression of 46,000 probes. Approximately 5500 transcripts are linked to one or more loci at a genome-wide $p < 0.05$. About 45% of these loci are located very close to the gene from which the mRNA is transcribed (so-called cis-acting quantitative trait loci; cis-QTLs). Another 55% of those loci are far from the gene location and are called trans-acting quantitative trait loci (trans-QTLs). Hundreds of transcripts often share common QTLs and these transcripts may often be part of overlapping molecular networks. This is the first set of transcriptome-QTL mapping data from the entire LXS RI strains, and the sample size is sufficient to study both main QTL effects and epistatic interactions. These valuable data can be used to study molecular networks related to diverse alcohol and stress phenotypes. Expression data and 122 matched classical phenotypes are available at www.GeneNetwork.org.

P53**HIDDEN LAYERS OF HUMAN SMALL RNAS**Hideya Kawaji¹, Mari Nakamura², Yukari Takahashi¹, Albin Sandelin³, Shintaro Katayama², Carsten Daub², Shiro Fukuda², Chikatoshi Kai², Jun Kawai², Jun Yasuda¹, Carninci Piero⁴, Yoshihide Hayashizaki^{1,2,4}¹Frontier Research System, RIKEN, ²Genomic Sciences Center (GSC), RIKEN, ³The Bioinformatics Centre, University of Copenhagen, ⁴Discovery Research Institute, RIKEN

Small RNA attracts increasing interest based on the discovery of RNA silencing and the rapid progress of our understanding of these phenomena. Although recent studies suggest the possible existence of yet undiscovered types of small RNAs in higher organisms, many studies to profile small RNA have focused on miRNA and/or siRNA rather than on the exploration of additional classes of RNAs.

We explored human small RNAs by unbiased sequencing of RNAs with sizes of 19-40 nt, and provided substantial experimental and computational evidences for the existence of independent classes of small RNA. Our data shows that well-characterized non-coding RNA are cleaved at sites specific to the class of ncRNA. We also found small RNAs mapped to genomic regions that are transcribed in both directions by bidirectional promoters, indicating that the small RNAs are a product of dsRNA formation and their subsequent cleavage. Our results underscore the complexity of the small RNA world and their production pathways.

P54**DEVELOPMENT OF 454-CAGE METHOD FOR MONITORING DYNAMIC GENE EXPRESSION CHANGE**

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We developed a CAGE (Cap Analysis Gene Expression) method to extract a short sequence from the 5' end of a transcript and successfully performed genome-wide gene expression analysis and promoter analysis with this method. However, the limitation of conventional Sanger sequence's capacity confined our analyses to "static" profiling of gene expression. Recent advent of ultra-high throughput sequencer such as a 454 sequencer promises to change the situation drastically and allows us to explore "dynamic" change on gene expression in time-course. To this end, our previous CAGE method has been adapted to 454 sequencer. In addition, we achieved to mix the CAGE tags from different time points by introducing "DNA tags" corresponding to each time point. Compared to the preparation of CAGE tags from each time point, the pooling tag approach using DNA tags improved the quality of gene expression profiling. Moreover, the pooling tag approach largely contributed to shorting sequencing time and saving sequencing cost. The improved CAGE method, 454-CAGE method, is believed to lead us to monitor the dynamic change on genome-wide gene expression.

P55**MATRIX RNAI AS A TOOL FOR DEPICTING A HUMAN INTER-TRANSCRIPTION FACTOR REGULATORY NETWORK**

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Transcriptional regulation between transcriptional regulatory factors (TRFs) and their genes is the center of gene expression control. To obtain an insight into the transcriptional regulatory network (TRN), we have applied the Matrix RNAi analysis in which a set of 19 TRF genes preferentially expressed in human hepatoma cells were individually knocked down by respective specific siRNAs. Perturbations in expressions of these and 2 additional cognate TRF genes were quantified. This approach detected 127 potential edge candidates (99 downregulated and 28 upregulated) in a total of 399 blocks of reciprocal regulation and various types of multicomponent regulatory motifs, which make up and support the robustness of the complex inter-TRF regulatory network. It also revealed the crosstalks of hepatocyte nuclear factors (HNFs) and CCAAT/enhancer-binding proteins (CEBPs), which play key regulatory functions in human hepatocytes or liver, with several nuclear receptors such as peroxisome proliferator activated receptors (PPARs), retinoic acid receptors (RARs), RAR-related orphan receptors (RORs) and retinoid X receptors (RXRs). Chromatin immunoprecipitation (ChIP) demonstrated that 70 % edges were both perturbation- and TRF binding-positive, depicting a validated functional TRN in the human hepatoma cells.

P56 TRANSCRIPTOME ANALYSIS BY 5'-3' DITAGS.

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Cap trapping gene expression (CAGE) used for the gene expression analysis and the profiling of transcriptional start points (TSP) including promoter usage analysis is the DNA tags deriving from the initial 20 nucleotides of 5' end mRNAs. We have developed the GSC (gene signature cloning) to obtain both 5' and 3' tags of the same RNA of subtracted cDNA using technology of CAGE and RIKEN Full-length cDNA. The structure of Ditag is that 3 ends of poly A is removed and there is a site to classify both of them between the 5' and 3' Tags. In particular, we have develop the technology in order to achieve tags of at least 20 nt for each side, which facilitate mapping operation and gene identification.

Therefore it is possible to understand gene structure correctly from transcription start site to termination site, and help mapping the CAGE tags that are mapping in multiple genomic locations. The various transcriptome patterns of the identical gene region are detected by analysis of GSC library sample. Furthermore the technique is used for high efficiency sequence analysis and cost reduction by using the 454 Life Sciences Sequencer.

P57 LOGIC OF REPEAT ELEMENTS TRANSCRIPTION

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The so called Junk DNA contains repetitive elements, which function has been debated. Conventional high-throughput expression profiling methods do not treat the expression of RNAs from repeat elements, due to the highly redundant nature of these sequences. By using cap-analysis gene expression (CAGE), a method based on short tags sequencing, we have identified that a very consistent fraction of the transcriptome transcribed by RNA polymerase II (5-10%) is constituted by repeat elements. We have analyzed the mouse and human transcriptome with CAGE and deep-CAGE. This analysis has revealed the following unexpected findings.

- (1) Repeat element are actively and pervasively transcribed by RNA pol II and different repeat families show different tissue specificity.
- (2) Expression of repeat elements is connected with the expression of single copy genes, and different loci show either positive or negative correlation with the presence of expressed repeats which map on their putative regulatory regions.
- (3) Repeat element expression is associated with specific gene families and GO terms.
- (4) Perturbations of expression of repeat elements cause specific cellular phenotypes.

We will discuss how these findings affect and complement our knowledge of transcriptional control and will provide models for the interpretation of the mechanisms of crosstalk between repeats-derived RNAs and the other RNA polymerase II transcribed RNAs.

P58**DECIPHERING THE PROMOTOME OF PURIFIED CELL TYPES WITH LONGER CAGE TAGS**

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Tag-based transcriptome analysis can detect the expression of many coding and non-coding transcripts, but in the case of functional analysis by comparison or time-course, their statistical power is concentrated on the small fraction of highly expressed genes. We circumvented this limitation by three improvements on the Cap Analysis of Gene Expression method (CAGE). First, we increased the size of the tags from 20 to 25 bp by using a different restriction enzyme, EcoP15I. This allows to rescue more than half of the multi-mapped tags, which are often discarded from further analyses. Second, we used a simpler method to capture 5'-complete ends, based on strand-switching. This allows to use much smaller samples, which is a requirement for analysing single cell type populations. These samples typically show a much less complex transcriptome than whole tissue samples. Third, we switched the protocol from Sanger sequencing to Solexa sequencing, which dramatically increased the number of tags for the statistical analysis.

P59**BIOINFORMATICS APPROACH TO IDENTIFICATION OF GENES INVOLVED IN MULTIPLE PITUITARY HORMONE DEFICIENCY: NOTCH SIGNALING AND ORTHODENTICLE RELATED TRANSCRIPTION FACTORS**

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Mouse studies have advanced understanding of the causes and mechanisms of pituitary hormone deficiency. Mutations in the transcription factors POU1F1 (PIT1) and PROP1 cause identical multiple pituitary hormone deficits arising from failed cell differentiation. Prop1 mutant pituitaries have failure of Notch gene expression, progenitor cell migration, enhanced apoptosis, poor vascularization, and dysmorphology. Pou1f1 mutant pituitaries lack these features, implying that Prop1 controls the expression of genes besides Pou1f1 that are important for pituitary cell migration, survival, and differentiation. We took several approaches to identify such genes, including microarray analysis of gene expression comparing pituitary transcripts from newborn Prop1 and Pou1f1 mutants and littermates. Approximately 17 biological process gene ontology terms are significantly over-represented amongst the gene expression changes unique to Prop1 mutants, including organ morphogenesis. A few genes with the most dramatically changed expression patterns were genes normally repressed by Prop1, including the Notch target Hey1 and the transcription factor Otx2. Otx1 and Otx2 have overlapping functions during development. Otx1 is necessary for normal pituitary hormone gene expression after birth, and Otx2 is required for normal pituitary gland shape. We confirmed elevated expression of Otx2 in Prop1 mutants in areas of pituitary dysmorphology arising from failed cell migration and differentiation. OTX2 mutations in humans cause a constellation of defects in head and brain development that can include pituitary hypoplasia. This demonstrates the effectiveness of this approach to identifying candidate genes for pituitary hormone deficiency of unknown etiology. Supported by NICHD, University of Michigan CCMB.

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A GENETIC ANALYSIS OF MELANOCORTIN 1 RECEPTOR (MC1R) GENE AND ITS RELATIONSHIP TO COAT COLOR IN A “MELANIC” VOLE (*Eothenomys melanogaster*)

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Melanic forms of animals have attracted recent interests of *Mc1r* gene and were often implicated in adaptive coloration shaped by natural selection. However, the individuals transmit genotypes, not phenotypes, to their offspring. Consequently, finding the link between phenotype and genotype for an adapted characteristic is an everlasting work in evolution. We sampled four populations of black-bellied vole (*E. melanogaster*) to analyze the *Mc1r* gene. This species catches our attention because the individuals are in general all “melanic” except some are less “melanic” by showing more pheomelanin in their hairs and thus giving a “brown” hue. This color polymorphism exists in the natural populations. Our results indicate the proportion of black individuals within each population increases with the increase of precipitation. The pattern agrees with the Gloger’s rule, implying the dark form could be adaptive associated with the amount of moisture in the habitats. We sequenced the exon of *Mc1r* in two populations, and found an association between phenotype (coat color) and genotype (*Mc1r* sequence) in a non-synonymous substitution. Furthermore, the polymorphic amino acid is at linkage disequilibrium with 2 other synonymous substitutions and a non-coding polymorphism, implying the change of the amino acid may be affected by natural selection. To explore further, we used 5’ rapid amplification of cDNA ends (5’RACE) to obtain the 5’UTR sequence of *Mc1r* gene and found some potential transcription factor binding sites. In the future, we plan to study the 5’ regulatory and coding region of *Mc1r* gene in all sampled populations.

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THE NATIONAL BIO RESOURCE PROJECT FOR THE RAT IN JAPAN - AN UPDATE

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Decades of broad research with experimental animals have lead to an enormous stock of biological resources. In particular rodents are being used to understand the function of human genes and pathways. Among them, rats and mice are the most important mammals used in various fields of medical and biological studies. Even though both species appear very similar, the rat is more suitable for many kinds of research due to its bigger size and different neurological characteristics.

The National Bio Resource Project for the Rat in Japan (NBRP-Rat) is now operating in its 2nd term. It was established in 2002 (1st term 2002-2006) to utilize existing laboratory rat resources. To achieve this goal, the workflow for this Bio Resource Project consists of the collection of rat strains, the cryopreservation of embryos and sperm, the preparation and maintenance of a publicly accessible database on the deposited rat strains and the global distribution of these rat strains. By August 2007, 355 rat strains are deposited to the NBRP-Rat, of which 163 strains are kept as live animals, 281 have already been preserved as embryos and 74 are stored as frozen spermatozoa.

6 male rats of 139 inbred strains are phenotypically characterized for 109 parameters, and 122 rat strains are genotyped with 357 microsatellite markers. Since 2006, the phenotyping for female rats has begun and data for 40 strains are already online. Further characterization is in progress and the permanent growing bilingual database on all deposited strains is accessible at <http://www.anim.med.kyoto-u.ac.jp/nbr>.

P62**GENOMIC ANALYSIS OF GASDERMIN A (GSDMA) AND GASDERMIN C (GSDMC) LOCI IN THE MAMMALIAN GENOME**

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We previously reported a novel gene family named Gasdermin (Gsdm), which is comprised of eight (Gsdma1, Gsdma2, Gsdma3, Gsdmc1, Gsdmc2, Gsdmc3, Gsdmc4 and Gsdmd) mouse genes and four (GSDMA, GSDMB, GSDMC and GSDMD) human genes. Gsdma and Gsdmc genes form clusters in the mouse chromosome 11 and 15, respectively, and are expressed in skin and gastrointestinal tract in a highly tissue specific manner (17th IMGC and Tamura et al., Genomics 2007). In this study, we carried out comparative genome analysis of the Gsdma and Gsdmc loci in mammals. Although mouse and rat belong to the same subfamily Murinae, rat Gsdma and Gsdmc genes are solitary in the genome. Moreover, among various species we examined, only the mouse genome appeared to contain clusters of Gsdma and Gsdmc genes. We found that number of clustering genes differ even among mouse strains derived from different subspecies. These results suggest that clustering of the Gsdma and Gsdmc genes are specific to the mouse genome, and the relevant gene duplication events occurred after the divergence of mouse and rat. Comparison of Gsdma and Gsdmc expression patterns between mouse and rat revealed that expression domain of the rat solitary gene is subdivided into expression domains of the mouse genes in the each cluster. Thus, all of the results suggested that Gsdma and Gsdmc clusters are one of the most useful subjects to analyze the gene duplication and the expression diversification during mammalian evolution.

P63**ANALYSIS OF GENE EXPRESSION RELATED TO THE ONE CARBON METABOLIC PATHWAY AND DNA METHYLATION STATUS IN THE RAT LIVER DURING VITAMIN B-12-DEFICIENCY.**

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During the regulation of gene expression, not only DNA base sequence but also epigenetic machinery takes place, and DNA methylation is considered as one of them. Vitamin B-12 (B-12) is an essential co-factor in one-carbon metabolic pathway, thereby ensuring the supplement of the methyl group donor, S-adenosylmethionine (SAM), to most biological materials including DNA. Whether the B-12 can alter specific gene expression through DNA methylation is still unsolved. Thus, to evaluate the gene expression change in the enzyme of one-carbon metabolism during B-12-deficiency, we established a severely B-12-deficient rat. Moreover, to examine the possibility whether dietary methionine supplement could complement B-12-deficiency, we gave 0.5% methionine to B-12-deficient rats and measured these genes mRNA level. The status of B-12-deficiency was confirmed by the increase of S-adenosylhomocysteine and decreases of SAM. We also found that the genomic DNA was hypomethylated. Quantitative-RT-PCR analysis revealed that the expression level of one-carbon metabolic pathway related enzymes mRNA changed in the liver of B-12-deficient rats. Most of these gene changes with B-12-deficient but recovered or showed tendency of recovery by 0.5% methionine or B-12 addition to B-12-deficient rats. However Cystathionine beta-synthase (Cbs) mRNA level decrease in B-12-deficient and did not recover by methionine addition. The DNA methylation status of CpG island of Cbs promoter was measured by bisulfite sequencing analysis and this area was hypomethylated in the B-12-deficient rats. These results demonstrate that B-12 can affect epigenetic machinery by regulating the DNA methylation status.

P64**TRANSCRIPTIONAL NETWORK ANALYSIS OF THE GLUCOCORTICOID RECEPTOR BASED ON THE HIERARCHICAL GENOMIC APPROACH**Masakuni Serizawa¹, Yoshinori Ochiai¹, Naoko Birukawa¹, Kazuyuki Yanai², Sabrina Jesmin¹, Norihiro Kato¹¹International medical center of japan, research institute, department of gene diagnostics and therapeutics²Toho university, faculty of science, department of biomolecular science, division of molecular medicine

Glucocorticoid elevates blood-sugar level by stimulating hepatic gluconeogenesis as well as by inhibiting the insulin secretion from β pancreas at cellular level. Hypersecretion of glucocorticoid is clinically known as Cushing's syndrome which pathologically simulates metabolic syndrome. And diabetic mellitus is a key component of metabolic syndrome. The present study aimed at revealing a relationship between glucocorticoid receptor (GR) and diabetes from the view point of genome-network analytical perspective. And for this purpose, it is crucial at first to systematically identify the candidate genes regulated by the direct binding of glucocorticoid receptor (GR).

Accordingly, the modified Yeast One-Hybrid (Y1H) was performed to explore the candidate region including the glucocorticoid receptor recognition element (GRE) on the rat genome sequence, and the putative GRE was screened by the MOTIF database search. Subsequently, we picked up 865 sequences including the putative GRE, and about 32% of them were mapped onto the intron. Then, the competition assay based on the "fluorescence correlation spectroscopy" was performed to clarify the direct interactions between the rat GR (rGR) protein and the randomly selected 101 putative GRE sequences. We found that GRE sequences of over 80 MOTIF-score interacted with rGR with a high probability. In addition, we performed microarray analysis in the cell lines of rat liver and pancreas to screen out the dexamethasone-responsive genes existing at the adjacent region of the 865 Y1H sequences. Thus, the present study is the first to report about 75 novel genes which apparently seem to be directly regulated by rGR.

P65**A NOVEL PEROXISOMAL PROTEASE TYSND1 IS REGULATED BY PPAR AGONIST AND PROCESSES PTS1- AND PTS2-CONTAINING ENZYMES INVOLVED IN β -OXIDATION OF FATTY ACIDS**Igor V. Kurochkin¹, Yumi Mizuno², Ken Yagi², Yuichi Ninomiya², Akihiko Konagaya¹, Yoshiyuki Sakaki¹, Christian Schönbach¹ and Yasushi Okazaki²¹RIKEN Genomic Sciences Center, Yokohama, Japan, ²Div. of Functional Genomics & Systems Medicine, Res. Ctr. for Genomic Medicine, Saitama Medical University, Saitama, Japan

Peroxisomes play an important role in β -oxidation of fatty acids. All peroxisomal matrix proteins are synthesized in the cytosol and post-translationally sorted to the organelle. Two distinct peroxisomal signal targeting sequences (PTSs), the C-terminal PTS1 and the N-terminal PTS2, have been defined. Import of precursor PTS2 proteins into the peroxisomes is accompanied by a proteolytic removal of the N-terminal targeting sequence. Although the PTS1 signal is preserved upon translocation, many PTS1 proteins undergo a highly selective and limited cleavage. Here, we demonstrate that Tysnd1, a previously uncharacterized protein, is responsible both for the removal of the leader peptide from PTS2 proteins and for the specific processing of PTS1 proteins. All of the identified Tysnd1 substrates catalyze peroxisomal β -oxidation. Tysnd1 itself undergoes processing through the removal of the presumably inhibitory N-terminal fragment. Furthermore, Tysnd1 expression is induced by the proliferator-activated receptor α (PPAR α) agonist bezafibrate, along with the increase in its substrates. A model is proposed where Tysnd1-mediated processing of the peroxisomal enzymes promotes their assembly into a supramolecular complex to enhance the rate of β -oxidation. Attempts to identify other putative substrates for Tysnd1 are also in progress.

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EXPLORATION OF ADIPOSE TISSUE TRANSCRIPTOME NETWORK TRIGGERED IN VIVO BY AN INSULIN SENSITIZING AGENT, PIOGLITAZONE

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Pioglitazone belongs to the class of thiazolidinediones (TZDs) that are ligands for nuclear receptor, peroxisome proliferators-activated receptor (PPAR) γ . PPAR γ modulates the expression of numerous genes involved in glucose and lipid homeostasis and plays a critical role in adipocyte differentiation. Considering substantial clinical impacts of PPAR γ on insulin resistance and related phenotypes, it is of interest to elucidate transcriptome network *in vivo* triggered by activation of PPAR γ . We therefore perform microarray analysis as well as Real-time PCR in rat to investigate gene expression changes induced by pioglitazone.

Gene expression changes were systematically evaluated with particular focus on adipose tissue after administrating pioglitazone in two inbred rat strains; spontaneously hypertensive rat and normotensive Wistar Kyoto rat, categorized broadly into two groups (n=4 each), with and without drug intervention for each strain. Adipose tissue was excised, and total RNA was extracted for subsequent DNA microarray (44K) analysis. Gene expression was quantitatively evaluated as fold-changes and finally inter-strain gene expression comparison in adipose tissue was made.

Although separately, a number of genes were differentially expressed in adipose tissue in both rat strains by treatment with pioglitazone compared to vehicle treatment group, some important genes appeared to be commonly altered and overlapped in adipose tissue by pioglitazone treatment in both strains. Some of these overlapping genes belong to adrenergic receptors and carbonic anhydrase such as *Adra2c*, *Car5*, *Car6* and so on. These findings suggest that pioglitazone modulates important gene expression in adipose tissue irrespective of blood pressure and insulin resistance in rat.

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CLONING, SEQUENCING AND QUANTIFICATION OF BCL-2 AND BAX GENES FROM LAGOSTOMUS MAXIMUS (Rodentia)

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South American caviomorph rodent *Lagostomus maximus* (Lm) displays exceptional and unique reproductive characters. Females ovulate between 600 to 800 eggs per reproductive cycle, representing the highest ovulation rate ever described. Besides having this massive ovulation, around 10 eggs are fertilized and implanted and only 1 or 2 embryos are gestated to term. The high ovulatory rate has been explained as a consequence of the highly convoluted anatomy of the ovary that increases the surface for ovulation. We have recently shown that massive ovulation arises from an over-expression of the apoptosis-inhibiting BCL-2 gene, a low expression of apoptosis-inducing BAX gene, and a highly suppressed apoptosis rate. This is an exceptional trait which contradicts the normal massive oocyte dismissal by apoptosis-dependent atresia that characterizes mammalian species. In this context, molecular characterization and sequencing of genes involved in this unusual mechanisms is a mandatory goal in order to further analyze the levels and meaning of BCL-2 over-expression and apoptosis suppression. We cloned and sequenced BAX and BCL-2 cDNA from Lm, obtained from total mRNA from different tissues of Lm. Sequences were used for designing specific primers and mRNA quantitation.

Both BCL-2 and BAX sequence showed to be highly conserved compared to other mammalian related species with >90% of homology. Surprisingly, BAX mRNA level was unspectly higher than BCL-2, contrasting with the low levels of protein detection in the ovary were BCL-2 was high expressed and BAX low or nule detected.

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THE RAT GENOME DATABASE: INTEGRATING BIOLOGICAL INFORMATION INTO THE GENOMIC CONTEXT

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The Rat Genome Database utilizes multiple biomedical ontologies for functional annotations of genomic and genetic elements not only to standardize data, but also to provide the user with a view of biological information in the context of the genome. Innovative tools such as GViewer and the Ontology Browser enable users to search for data based on their biological properties. The Ontology Browser allows the user to search multiple vocabularies simultaneously, and to retrieve multiple types of data including genes, QTLs and strains, annotated both with the chosen term and with terms of greater specificity. The genes, QTLs and strains retrieved provide a link between biological properties such as disease association or phenotype, participation in a molecular pathway or having a molecular function, and mapped locations on the genome. Ontology reports include the GViewer which allows users to see a genome-wide view of a particular biological property, download that data for use in other applications, or navigate to GBrowse for a closer look at the genome. GBrowse provides detailed information on the genomic environment, allowing users to visualize data such as overlapping QTLs or the genes and SNPs localized within a QTL. An additional tool, VCMMap also links disease and phenotypes to the genome across species through its multiple QTL maps. RGD's data presentation tools facilitate integration of biological information into a genomic context.

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CELL DEATH WITH EXCESSIVE AUTOPHAGY OCCURS IN SERINE PROTEASE INHIBITOR KAZAL TYPE 3 DEFICIENT ACINAR CELLS

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Background & Aims: Serine protease inhibitor Kazal type 1 (SPINK1) is thought to inhibit trypsin activity and to prevent pancreatitis. Point mutations in the *SPINK1* gene seem to predispose humans to pancreatitis; however, the clinical significance of *SPINK1* mutations remains controversial. This study aimed to elucidate the role of SPINK1.

Methods: We generated *Spink3* (mouse homologue of SPINK1) -deficient (*Spink3*^{-/-}) mice by gene targeting in mouse embryonic stem cells. Embryonic and neonatal pancreases were analyzed morphologically and molecularly. Specific probes were used to show the typical autophagy that occurs during acinar cell death.

Results: In *Spink3*^{-/-} mice, the pancreas developed normally up to 15.5 days after coitus. However, autophagic degeneration of acinar cells, but not ductal or islet cells, started from day 16.5 after coitus. Rapid onset of cell death occurred in the pancreas and duodenum within a few days after birth and resulted in death by 14.5 days after birth. There was limited inflammatory cell infiltration and no sign of apoptosis. At 7.5 days after birth, residual duct like cells in the tubular complexes strongly expressed pancreatic duodenal homeodomain-containing protein 1, a marker of pancreatic stem cells, without any sign of acinar cell regeneration.

Conclusions: The progressive disappearance of acinar cells in *Spink3*^{-/-} mice was due to excessive autophagy and impaired regeneration. Thus, *Spink3* has essential roles in the maintenance of integrity and regeneration of acinar cells.

P70**AUTOPHAGY DELIVERS TRYPSINOGEN TO THE LYSOSOME IN ACUTE PANCREATITIS**

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Background & Aims: Auto(self)-digestion of the pancreas by its own prematurely activated digestive proteases is thought to be an important event in the onset of acute pancreatitis. Although lysosomal cathepsin B is suggested to play a key role in intrapancreatic trypsinogen activation, it is not clear how trypsinogen is delivered to the lysosome. Autophagy is an intracellular bulk degradation system in which cytoplasmic components are directed to the lysosome/vacuole by a membrane-mediated process. The purpose of this study is to analyze whether autophagy delivers trypsinogen to the lysosome and whether this is related to onset of acute pancreatitis.

Methods: We produced a conditional knockout mouse lacking *Atg5* (autophagy related gene 5) in pancreatic acinar cells.

Results: Severity of acute pancreatitis induced by cerulein was greatly reduced in these mice. In addition, trypsin activation was not observed in *Atg5* deficient acinar cells. These data suggest that autophagy exerts a devastating effect in pancreatic acinar cells by activation of trypsinogen to trypsin.

Conclusions: We propose that autophagy plays a major role in pathogenesis of acute pancreatitis by delivering trypsinogen to the lysosome.

P71**DEVELOPMENTAL DEFECTS OF KIDNEY IN FSTL1 GENE TARGETING MICE**

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Congenital malformations of the urinary tract are a major cause of renal failure in children and young adults. However, the underlying molecular and cellular mechanisms are poorly understood. *Fstl1* (follistatin-like 1, TSC-36, FRP) is a secreted extracellular glycoprotein, which modulates the activities of BMPs or TGF- β related ligands as an inhibitor of TGF- β superfamily in a way similar to follistatin. Here, we report the phenotypic characterization of congenital ureter malformation in *Fstl1* conventional knockout mice. *Fstl1* is expressed in mesenchymal cells surrounding the ureter stalk. From E16.5, kidneys of *Fstl1*^{-/-} mice displayed a prominent hydroureter, which was usually more severe in the proximal region compared with wildtype or heterozygous littermates, whereas no physical blockage along the ureter was observed. In *Fstl1*^{-/-} mice, the ureteral mesenchymal and epithelial cells showed reduced proliferation by Brdu incorporation counts. Moreover SMA (smooth muscle α -actin)-producing smooth muscle cells and pan-Cytokeratin-producing epithelial cells were also reduced. *Fstl1*^{-/-} kidneys developed a hydronephrosis phenotype until E17.5, while the gross anatomy and histological structure of *Fstl1*^{-/-} kidneys was not affected in earlier stages. Our results suggest an important role of *Fstl1* in ureter development and *Fstl1*^{-/-} mice can be a novel mouse model for congenital ureter malformation.

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MOUSE KIDNEY PROXIMAL TUBULE-CELL SPECIFIC ABLATION MEDIATED BY HUMAN DIPHTHERIA TOXIN RECEPTOR

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Human heparin-binding EGF like growth factor (HB-EGF) can bind diphtheria toxin (DT) at high affinity and is termed as diphtheria toxin receptor (DTR), but mouse HB-EGF does not bind. By introducing human HB-EGF gene under a tissue-specific promoter, we can produce a transgenic mouse in which a conditional and targeted cell ablation occurs. *Gs15* (glycosphingolipid controlling gene-5) regulates the expression of a glycolipid and glycoproteins that contain the Le^x epitope in mouse kidney through tissue-specific transcriptional regulation of the core 2 b-1,6-*N*-acetylglucosaminyltransferase (c2GnT). We have identified it as a *cis*-regulatory element responsible for proximal straight tubule cell-specific transcription. Utilizing the revealed sequence of *Gs15*, we generated transgenic mice that express human HB-EGF in their kidney proximal straight tubule cells. RT-PCR analysis confirmed that the transgenic (B6-*Gs15*-TRECK Tg) mice express mRNA of human HB-EGF only in kidney. Administration of DT into Tg mice showed a dose-dependent depletion of proximal tubular cells, proteinuria as the result of excretion of serum albumin, and an increase of the blood urea nitrogen (BUN) value. In histological study, the most severe injury was observed in straight tubules in which tubular cells are exfoliated into tubular lumina. The straight tubule portion is known to be the most sensitive to ischemic injury and to have a remarkable capacity of regeneration. The B6-*Gs15*-TRECK Tg mice are a useful model for the elucidation of the mechanism of acute renal failure and regeneration of tubular cells.

P73

EPITHELIAL-SPECIFIC BLOCKAGE OF THE MYD88-DEPENDENT TLR SIGNALING PATHWAY CONTRIBUTES TO SPONTANEOUS INTESTINAL INFLAMMATION

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To elucidate the potential role of epithelium-intrinsic myd88-dependent Toll-like receptor (TLR) in the pathogenesis of inflammatory bowel disease (IBD), a transgenic mouse model in which the flag-epitope tagged dominant-negative Myd88 (dnMyd88) driven by a 12.4kb *villin* promoter was generated. Expression analysis revealed that the transgene was specifically expressed in the epithelium of small intestine and colon. At 6 weeks, the transgenic mice demonstrated enlargement of the small intestine, increased crypt depth and width, and significantly increased proliferation compartment (BrdU positive cells/crypt, 20.6 vs 6.2, $P < 0.01$). By the age of 48 weeks, the transgenic mice showed obvious phenotype of small intestinal inflammation, including increased inflammatory cells infiltration, crypt abscess, crypt branching, and elevated inflammatory cytokine levels. Alcian-Blue staining revealed that the number of goblet cells were significantly decreased (A-B positive cells/crypt, 6.11 vs 14.44, $P < 0.01$), but real-time quantitative PCR did not detect any change in MUC2 mRNA expression in transgenic mice. TUNEL assay also revealed increased apoptosis in transgenic mice. Further more, we examined the possible mechanism of intestinal inflammation. Real-time quantitative PCR revealed that expression of β -defensin 3 and claudin-1,4,7 levels did not change significantly in transgenic mice. However, the Paneth-cell-derived α -defensins (also named cryptdins) were significantly decreased (general cryptdins, relative mRNA levels, 1.0 vs 0.33, $P < 0.05$).

Our study identified that myd88-dependent TLR signaling in the intestinal epithelium was a critical regulator of epithelial defense and intestinal immuno-homeostasis, and the results might provide important implications for understanding the possible mechanisms controlling the pathogenesis of human IBD, such as Crohn's disease.

P74**EMMA - THE EUROPEAN MOUSE MUTANT ARCHIVE**

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The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. A full description of these services can be viewed on the EMMA website (<http://www.emmanet.org>).

The EMMA network is comprised of seven partners who operate as the primary mouse repository in Europe and is funded by the participating institutes and the European Commission Research Infrastructures Programme.

EMMA's primary objectives are to establish and manage a unified repository for maintaining mouse mutations and to make them available to the scientific community. In addition to these core services, the consortium can generate germ-free (axenic) mice for its customers and also hosts courses in cryopreservation.

All applications for archiving and requests for mutant mouse strains are submitted through the EMMA website. Mouse strains submitted for archiving are evaluated by EMMA's external scientific committee. Strains held under the EMMA umbrella can be provided as frozen materials or re-derived and shipped as live mice depending on the customer's needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery. All animals supplied by EMMA are classified as SPF in accordance with the FELASA recommendations.

EMMA is a founding member of FIMRE (International Federation of Mouse Resources) and actively cooperates with other leading repositories like TJL and the MMRRC in the US and BRC RIKEN from Japan.

P75**FBXO11, MUTATED IN OM MOUSE MUTANT MODEL JEFF, BINDS THE SMAD3/SMAD4 ANCHOR PROTEIN SPECTRIN BETA II**

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The Jeff mutant carries a mutation in the F-box gene, Fbxo11, a member of a large family of proteins that are specificity factors for the SCF E3 ubiquitin ligase complex (Hardisty-Hughes R. et al. 2006 Hum. Mol. Genet. 15, 1-7). Jeff develops a conductive deafness due to a chronic suppurative otitis media and represents an important model for chronic forms of middle ear inflammatory disease in humans. We have transfected both wild type and F-box deletion (delF11) forms of Fbxo11 into cos7 cells. We demonstrated that over-expression of delF11 causes severe growth inhibitory effects in contrast to overexpression of wild type Fbxo11. Further analysis of apoptosis among transfected and untransfected cos7 cells strongly indicates that Fbxo11 has anti-apoptotic effects. These results agree with recent findings which demonstrate that Fbxo11 neddylates and inhibits p53 which itself is pro-apoptotic. In addition, we have proceeded to identify interacting partners to Fbxo11 and to establish potential substrates for ubiquitination mediated by Fbxo11. We have performed co-immunoprecipitations with Fbxo11 from both cos7 and mouse tissue and identified spectrin beta II as an interacting partner to Fbxo11. Spectrin beta II is known to interact with Smad3 mediating the TGF- β signalling pathway. The interaction of Fbxo11 with spectrin beta II provides in vivo evidence for the pivotal nature of this signalling pathway in mediating otitis media and suggests that Fbxo11 may mediate OM through two routes - the TGF- β signalling pathway and via the pro-apoptotic effects of p53.

P76**WHICH C57BL/6 SUBSTRAIN IS USED FOR THE BACKGROUND STRAIN OF YOUR MOUSE?**

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The C57BL/6 (B6) is the most well-known inbred mouse strain established in the 1920s, and has widely been used as the genetic background of congenic and mutant mice. A number of B6 substrains including C57BL/6J (B6J) and C57BL/6N (B6N) have been derived from the B6 founder line and there are differences among them for several genes and phenotypes. In Japan several B6 substrains, such as B6J (JAX), B6JJcl, B6NJcl and B6CrSlc are available. The importance of these substrains differences, however, has not yet been well recognized among biomedical researchers. Here, we report which B6 substrains were used as the background of congenic and mutant strains deposited in RIKEN BRC that has been designated as a central core facility for the mouse resources in Japan, and the results of comprehensive SNPs analyses using 1,446 loci among B6 substrains available in Japan. According to information provided by depositors, 374 B6 background strains were classified as follows: 63% were crossed with the B6J, 11% with the B6N and 11% with B6CrSlc. The other 15% were of mixed background among B6 substrains or of uncertain B6 substrain. SNPs analyses clearly demonstrated genetic differences between B6J and B6N at several loci. The B6CrSlc available from Japan SLC originally derived from the NCI-Frederick was found to be the same as the B6N by the SNPs pattern. Moreover, we found a single SNP between B6J (JAX) and B6JJcl (from CREA Japan). These data will be useful for accurate genetic monitoring among genetically-engineered mice with the B6 background.

P77**LASER-ASSISTED IVF - AN ALTERNATIVE APPROACH FOR SUCCESSFUL CRYOPRESERVATION OF MUTANT MOUSE LINES ON C57BL/6 BACKGROUND**

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For mutant mouse strains on C57BL/6 background, embryo freezing - preferably after in vitro fertilization (IVF) using fresh sperm of a mutant male and wildtype oocytes (=Cryo-IVF) - is the routine cryopreservation method, because frozen-thawed sperm of these strains has a poor fertilizing ability. A quite high number of oocyte-donors (wildtype females), however, are necessary to reach the necessary number of embryos.

We report here the successful archiving of 14 mouse mutant lines (targeted, gene-trap, transgenic) backcrossed 4-20 generations to C57BL/6 and submitted to the European Mouse Mutant Archive (www.emmanet.org) comparing two different methods. 1. Cryo-IVF; a part of the frozen embryos was thawed and transferred to foster mothers. 2. Laser-IVF, using frozen-thawed sperm and zona-dissected C57BL/6 oocytes (OctaxTM 1.48 µm laser, hole size 8µm). For Control-IVF, frozen-thawed sperm and untreated oocytes were used. The IVF-embryos were partly transferred to foster mothers.

As a result, all 14 lines could be preserved using both methods. Whereas for the Cryo-IVF 135 females per line were needed to produce on average 644 embryos, for the Laser-IVF only 15.4 females were needed to produce 87.3 embryos. The Laser-IVF cleavage rate was significantly higher compared to the Control-IVF (52.2 vs. 16.3%, $p < 0.001$). However, the birth rate after Laser-IVF (11.4%) was significantly lower ($p < 0.001$) compared to Cryo-IVF (34.7%) or Control-IVF (32%). In conclusion, sperm freezing and Laser-IVF are an alternative approach for cryopreservation of valuable mouse mutant lines on C57BL/6 background. Compared to Cryo-IVF, less animals are needed which contributes to animal welfare and general cost savings.

P78**SCREENING FOR DYNEIN COMPLEX MUTATIONS IN AN ENU MOUSE LIBRARY**

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Degenerative disorders of motor neurons include a range of progressive fatal diseases such as amyotrophic lateral sclerosis (ALS), spinal-bulbar muscular atrophy (SBMA) and spinal muscular atrophy (SMA). Although the molecular basis of neurodegeneration in these disorders remains unknown, an impairment in neuronal transport has been suggested to play a role in this process. Phenotype driven mutagenesis studies are an effective method to generate mouse models of such diseases and identify candidate genes for further study. One such study has identified the /Loa/ mice, which carry a point mutation in the dynein heavy chain causing a progressive locomotor disorder.

The cytoplasmic dyneins are large multi-component molecular motors involved in retrograde transport along microtubules and function in many cellular processes (e.g. vesicular transport, mitosis). The study of the *Loa* mice also suggests that the dyneins may contribute to neuronal survival. While the *Loa* mutation lies within the dynein subunit responsible for motor activity, it is not yet known whether mutations in the other dynein chains may also shed light on the molecular basis of motor neuron degeneration.

Here we present an initial screen of Harwell archive of ENU mutated mouse DNAs, looking specifically for mutations in the dynein complex. We have identified three different dynein mutants, each carrying a change to an amino acid highly conserved amongst vertebrates. It was found that these mutations fall in known binding domains suggesting that they may impair complex assembly. We intend to study rederive these mice in order to better understand the effects mutations in dynein have on the animal's phenotype.

P79**DEAFNESS MOUSE MUTANTS WITH HAIR CELL IMPAIRED CALCIUM-SIGNALING GENERATED BY THE RIKEN ENU MUTAGENESIS PROGRAM**

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RIKEN GSC

The development of mouse models would greatly increase our understanding of the mechanisms underlying hearing impairment, besides facilitating progress in current methods of diagnosis and treatment of this disorder. In order to develop a novel human deafness model in mouse, we used an ENU-mutagenesis screening platform to identify mice with hearing impairments that are relevant to those of human hearing disorders. 25 G1 were isolated to show deafness phenotype that were clearly inherited by the G2 generation. The phenotype was not accompanied by other traits, a characteristic of human diagnostic-type nonsyndromic deafness. Among the 25 lines, 4 lines were mapped to the distal region of chromosome 6 and one of the 4 was confined to the 7Mb region of Chr6 containing the *Pmca2* gene, suggesting that this gene might be causative. Direct sequencing revealed all these 4 lines harbored missense mutation in *Pmca2* gene and one of 4 had single amino acid substitution within the substrate-binding domain of *Pmca2*, indicating that it is probably defective in hair cell calcium-ion signaling. Since all the 4 lines showed different extent of deafness, they may constitute new alleles of the *Pmca2* gene with modification of the gene functions in different extent. Other 8 lines out of 25 were mapped to the region of Chromosome 10, and inferred to be mutants of *Cdh23*. The phenotypic variations will be discussed in reference to current framework of our understanding of the function of this gene and its relevance to human hearing disorders.

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THE JACKSON LABORATORY REPOSITORY: RESOURCE FOR INDUCIBLE AND CONDITIONAL MUTATION MOUSE STRAINS FOR MODELING HUMAN DISEASE

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The Jackson Laboratory

The Jackson Laboratory Repository serves as a resource for the scientific community, importing, developing, preserving and distributing mouse strains vital to biomedical research. Every year 200 to 300 new strains are added to the approximately 3,000 strains and stocks of mice currently held in the Repository collection. Whether harboring a traditional knockout targeted mutation, expressing a transgene, or utilizing recombinase technology, reporter/fluorescent proteins, or Tet-On/-Off technologies, donating investigators have provided numerous new strains with applications for modeling human disease. The Repository contains various reporter and lineage marker stocks including strains that express variants of fluorescent proteins with distinct detectable fluorescent signals allowing multiple labeling experiments. For non-invasive in vivo imaging studies, the Repository distributes mice that carry the luciferase gene. Mutant strains with inducible expression of reporter molecules allow researchers to control expression in a temporal and/or tissue-specific fashion. Site specific recombinase (such as Cre) expressing strains used in conjunction with conditional (loxP-flanked) mutants enhances the ability to study gene function in vivo. An online resource (www.jax.org) allows researchers to retrieve information related to strains maintained in the Repository. On-line strain datasheets typically include a brief phenotype description, strain development, strain maintenance procedures, licensing requirements and a list of related references. Donating a strain to the Repository fulfills the requirements for sharing of mice in accordance with NIH's policy for the sharing of research reagents. Researchers wishing to have strains considered for inclusion in the Repository are encouraged to submit their strains using the form available at The Jackson Laboratory website: <http://www.jax.org/grc/index.html>. The Jackson Laboratory Repository is supported by the NCRR (RR09781, RR11083, RR16049), NIA, The Howard Hughes Medical Institute, The Ellison Medical Foundation and donations from several private charitable foundations.

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A MOUSE MODEL FOR JUVENILE HYDROCEPHALUS

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Juvenile hydrocephalus is a neurological disease that affects 1 in 1000 human children. Hydrocephalus results from increased cerebral spinal fluid (CSF) in the ventricles of the brain, and may be due to overproduction, decreased removal, or a blockage to flow. A significant percentage of hydrocephalus is believed to be genetic in nature, yet little is known about genetic causes of the disease. Animal models for hydrocephalus, which are genetically tractable, can prove valuable in identifying candidate genes.

The *Jh* (juvenile hydrocephalus) mouse line carries a transgenic insertion on proximal chromosome 9. Mice homozygous for the insertion develop hydrocephalus by 1-2 weeks of age, and few survive beyond 8 weeks, indicating that the integration altered a gene involved in CSF balance. None of the known hydrocephalus mutations map to the *Jh* chromosomal region, so the *Jh* animals represent a new model for juvenile hydrocephalus. Characterization of the *Jh* integration site has shown that the transgene disrupts an uncharacterized gene called *I11*, producing an *I11-lacZ* fusion RNA that is likely a functional null. Preliminary evidence indicates that a transgene carrying the *I11* gene can rescue the *Jh* phenotype, supporting *I11* as the causative gene. The predicted I11 protein has no recognized functional domains, and the role it may play in CSF maintenance is unknown. Characterization of the *Jh* mice, and functional investigation of the I11 protein, will provide information about CSF balance and the etiology of juvenile hydrocephalus.

P82**GENE TARGETING IN C57BL/6 ES CELL LINES: EFFECTS OF GENETIC VARIATION AND CHROMOSOMAL INSTABILITY**

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We assessed genetic variation in six C57BL/6 mouse ES cell lines with 275 SSLP markers and compared the results to C57BL/6J reference DNA. The Bruce4 and CMT1-2 ES cell lines were the most divergent from C57BL/6J DNA, differing at 34 SSLP markers and showing significant regions of heterozygosity on chromosomes 3, 7, and 9. The BL/6#3 and Dale1 ES cell lines were the most similar to C57BL/6J, differing only at 3 SSLP markers. The C-2 and WB6d ES cell lines differed from C57BL/6J at 6 SSLP markers. No C57BL/6 ES cell line was genetically identical to C57BL/6J. One factor reducing gene targeted mouse production efficiency is chromosomal instability. We counted chromosome spreads from 820 gene targeted ES cell clones and subclones and found the Bruce4 C57BL/6 ES cell clones were more likely to be aneuploid and unsuitable for ES cell-mouse chimera production than 129 mouse derived ES cell clones. We derived the Bruce4.G9 subclone from Bruce4 ES cells and found that they have improved genetic stability (less tendency to aneuploidy) but lower plating efficiencies after electroporation with gene targeting vectors. Transfer of mutations engineered in Bruce4 ES cells to a defined C57BL/6J background, despite segregating alleles, is more rapid than transferring changes engineered in 129 mouse ES cells. The benefits of analyzing mutant phenotypes in a defined genetic background can offset the inefficiency of gene targeting in C57BL/6 ES cells.

P83**MOUSE EMBRYO/SPERM BANK AT THE CENTER FOR ANIMAL RESOURCES AND DEVELOPMENT (CARD), KUMAMOTO UNIVERSITY**

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In CARD Kumamoto University, embryo/sperm bank has been managed for the maintenance of mouse strains as genetic resources since 1998. Our essential services are: 1) Collection: We have collected about 1300 mouse strains including mutant and genetically engineered mice. 2) Cryopreservation: We generally produce embryos from these mouse strains using in vitro fertilization, and spermatozoa and 300 two-cell embryos were cryopreserved using the vitrification method in each strain. 3) Database: The information of 578 strains that we have cryopreserved is available as an online database in CARD R-base (<http://cardb.cc.kumamoto-u.ac.jp/transgenic/index.jsp>), and this information can be also searched in IMSR (International Mouse Strain Resource, <http://www.informatics.jax.org/imsr/index.jsp>). 4) Quality control: The quality of cryopreserved embryos is tested by the development of embryos to normal offspring after embryo transfer, and subsequent microbial monitoring. 5) Supply: These cryopreserved embryos or populations derived from cryopreserved embryos are supplied to the scientific community. We have supplied 64 strains of cryopreserved embryos and 168 strains of populations worldwide.

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NOVEL MOUSE MODELS OF DIABETES MELLITUS GENERATED BY THE RIKEN ENU MUTAGENESIS PROJECT

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Riken GSC

In RIKEN mutagenesis project, to generate mouse models for various human diseases, including lifestyle-related diseases, we have screened for many different phenotypes in the mutated animals. The number of mutants opened to the public on our website stands at more than 300 (<http://www.gsc.riken.go.jp/Mouse/>).

We report here a series of new mouse models for human diabetes. We screened approximately 12,000 G1 animals for early-onset dominant traits using a clinical biochemical test, then isolated hyperglycemic outliers. Using their N2 progeny, we confirmed the inheritance of the phenotypes and performed OGTT and ITT for initial characterization. Whole genome and fine mapping analyses with SNPs and microsatellite markers revealed some mutations were linked within several-kb regions on some chromosomes. Candidate gene approaches of these fine-mapped lines identified 12 glucokinase (*Gck*) mutants, 3 Insulin receptor (*Insr*) mutants, and one Single-minded homologue 1 (*Sim1*) mutant.

These ENU mutagenesis-derived mutants are highly useful models to analyze the mechanism of human type2-diabetes onset and progression. First, the *Gck* mutants showing decreased glucose-responsive insulin secretion can be the model of the early stage of type2 diabetes, especially for Asian type2 diabetic patients, as well as the MODY2 model. Second, the *Insr* mutants showing hyperinsulinemia and hyperglycemia can be the model of insulin resistance caused by impaired insulin signaling. Finally, the *Sim1* mutants, showing early-onset obesity, hyperinsulinemia and hyperglycemia, can be the model of diabetes mediated by obesity-related insulin resistance.

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A NOVEL PHEX MUTATION WITH DEFECTIVE GLYCOSYLATION CAUSES HYPOPHOSPHATEMIA AND RICKETS IN MICE

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N-ethyl-N-nitrosourea (ENU) mutagenesis is a phenotype-driven approach with potential to assign function to every locus in the mouse genome. We generated an ENU-based new mutation, *Pug*, as a mouse model for X-linked hypophosphatemic rickets (XLH) in human. Mice carrying the *Pug* mutation exhibit abnormal phenotypes including growth retardation, hypophosphatemia and decreased bone mineral density (BMD). The new mutation was mapped to X-chromosome between 65.4cM and 66.6cM, where *Phex* gene resides. Sequence analysis revealed a unique T-to-C transition mutation resulting in Phe-to-Ser substitution at amino acid 80 of PHEX protein. In vitro studies of *Pug* mutation demonstrated that PHEX^{pug} was incompletely glycosylated and sequestered in the endoplasmic reticulum region of cell, whereas wild-type PHEX could be fully glycosylated and transported to the plasma membrane to exert its function as an endopeptidase. Taken together, the *Pug* mutant directly confirms the role of *Phex* in phosphate homeostasis and normal skeletal development and may serve as a new disease model of human hypophosphatemic rickets.

P86**A BREEDING SCHEME FOR THE FUNCTIONAL ANALYSIS OF THE MOUSE X CHROMOSOME**

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Functional analysis of the mouse genome via large-scale mutagenesis programs has yielded numerous new autosomal dominant and recessive mouse mutants, but X-linked phenotypes have been largely underrepresented in these screens, and X-linked recessive conditions have been virtually nonexistent. We have therefore devised a breeding scheme to detect induced X-linked recessive phenotypes. Male mice are mutagenized with the chemical mutagen ENU and then bred to XO female mice. Some of the mice that result from this cross receive a mutagenized X chromosome from their father and no sex chromosome from their mother. These mice are therefore hemizygous XO females and will express any X-linked recessive phenotype that was induced by the mutagenesis. Mutant mice are identified by a phenotype screen, which includes blood and urine analyses, and are bred to determine both heritability and X-linkage of the mutant phenotypes. Thus far, we have used the above scheme to generate 25 mice that have inherited a mutagenized X chromosome from their father and no second sex chromosome from their mother. Even among this small set of animals, a number of abnormal phenotypes have been detected. We are currently breeding these animals to test for heritability of these phenotypes, and we are generating more mutagenized animals at an exponential rate for further study. Many of the resulting mutants are likely to be models of human X-linked conditions, thus providing insights into the cause and pathophysiology of X-linked inherited diseases.

P87**CHARACTERIZATION AND MAPPING OF BARTHEZ, A NEW MOUSE MUTATION WITH A COMPLEX PHENOTYPE THAT INCLUDES ALOPECIA**

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In this study we present barthez (bar), a new autosomal recessive mutation with a complex phenotype that includes growth retardation and hair loss. The initial mapping using F2 mice allowed us to localize the bar locus to chromosome 2, between markers D2Mit153 and D2Mit367 (29.2 - 33.4 Mb), a region of homology with human chromosome 9q34.1. The phenotype of bar/bar mice is obvious a few days after birth and includes severe retardation in post-natal development, alopecia with scaly skin, ataxia and circling behavior. A majority of the mutants die before weaning. Histologically, the mutants are characterized by a broad range of pathological alterations in multiple organs. Some of these alterations may be secondary to growth retardation (e.g. delayed ossification; delayed sexual maturity), however others appear to be specific, such as skin hyperkeratinization and hair follicle dysplasia, kidney and liver degenerative changes, and liver hemosiderosis. Other histological findings include atrophic changes in cerebellum, pancreas, and salivary glands, as well as muscular dystrophias. Collectively, this complex phenotype is reminiscent of the symptomatology associated to the syndrome of human congenital disorders of glycosylation (CDG). Positional and functional candidate genes for the bar mutation include Dolk (dolichol kinase), Dolpp1 (dolichyl pyrophosphate phosphatase 1), and Dpm2 (dolichol-phosphate (beta-D) mannosyltransferase 2).

P88**ENU-INDUCED RANDOM MUTAGENESIS SCREEN FOR IMMUNE OR ALLERGIC DISEASE MODEL IN THE MOUSE**

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To establish allergic and immune disease model animals, we conducted N-ethyl-N-nitrosourea (ENU) random mutagenesis into mice together with various immunological screen. In this presentation, recent result of non-sensitized and sensitized ENU mutagenesis screen will be shown. We injected ENU into C57BL/6 male and obtained the next generation male mice each as the independent founders, and obtained the third next generation to produce the recessive mutant pool of each pedigree. Since we produced every mutant generation by in vitro fertilization and embryo transfer, we could easily obtain a large number of mice of each pedigree at the same developmental stage. Mice were screened morphological and behavioral anomalies, hematological alterations and immunological defects at 12 and 16 weeks of age. To date, we obtained 64 pedigrees, 3526 G3 progeny and screened all mice for visible mutations. Furthermore 2063 mice (1009 female and 1054 male) in this ENU mutant mice pool were investigated for non-sensitized blood-based parameters in three years of our RIKEN RCAI ENU mutagenesis project. As a result, we have isolated 70 mutant lines, including such as allergy symptoms (1 pairs), abnormal number of white blood cells (14 pairs) and increased amount of serum immunoglobulin (48 pairs). And we have set up a new sensitized screen using the ENU G3 mice in order to identify enhancing or suppressing factor of pollen induced allergy, which lots of Japanese afflict with. From this screening, 6 mutant lines were isolated, and these mice are now under heredity confirmation.

P89**ICST IS A MOUSE MODEL FOR GLAUCOMA**

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ENU mutagenesis is a powerful tool for revealing gene function. We are studying an ENU-induced mutation called iris-corneal strands (*lcst*) that causes raised intraocular pressure, bulging eyes and anterior segment scarring when heterozygous and is recessive lethal. *lcst* homozygotes have limb abnormalities, absent cerebellum and skull defects. *lcst* is a missense mutation of the LIM-homeodomain transcription factor *Lmx1b* that abrogates the ability of the mutant protein to bind to its target DNA sequence. Mutations in *LMX1B* underlie the dominant human disorder nail-patella syndrome. This is a pleiotropic disorder characterised by nail and joint dysplasia sometimes accompanied by kidney defects and glaucoma. There is great variability in the disease aetiology both within and between families carrying different mutations in *LMX1B* and it is thought to be due to haploinsufficiency. *Lmx1b* has been knocked-out in the mouse and although the reported homozygous phenotype is very similar to what we find with *lcst*, heterozygous knock-out mice are reported to be normal. This contrasts with the strongly penetrant eye phenotype of *lcst* heterozygotes. Both mutant models are on C57BL/6 indicating strain differences are unlikely to account for the observed phenotypic differences. *lcst* is a model for glaucoma and will be useful in assessing treatments for this disease. This is the first instance where a mutation in the same endogenous gene gives the glaucoma hallmarks of intraocular pressure elevation and optic nerve excavation in both mouse and human. This work demonstrates the value of generating more than one mutant allele of a gene.

P90**EGTC, DATABASE FOR THE EXCHANGEABLE GENE TRAP CLONES; RESOURCE OF MOUSE AND ES CELL LINES FOR THE FUNCTIONAL ANALYSIS OF THE MOUSE GENOME**

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Gene trapping in ES cells is a proven method for large-scale random insertional mutagenesis in the mouse genome. Gene trap vectors contain a promoter-less reporter gene downstream of a splice acceptor and a selectable marker gene. A fusion transcript between the integrated (trapped) gene and the reporter gene can be easily cloned by 5'-RACE. We have developed exchangeable gene trap vectors, pU18, pU21, pU21B, pU21T and pU22. The reporter β -geo gene can be exchanged into any other DNA of interest through Cre-mediated recombination. We have isolated trap ES clones, determined sequence tags (GSSs) of trapped genes by 5'-RACE, and annotated them by BLAST and BLAT search. The data of trap clones are opened as a Database for the Exchangeable Gene Trap Clones, EGTC (<http://egtc.jp>). Until the end of July 2007, total 310 GSSs have been registered. Among them, 73% are known genes, 19% are ESTs, and 8% are new genes. 249 GSSs were determined their chromosomal localization through BLAT search. Since the pU21, pU21B and pU21T vectors have stop codons in upstream of the start codon of the β -geo, the integration sites of the vectors showed a strong bias to the vicinity of the ATG exon of trapped genes, indicating that these vectors could induce a null allele efficiently. In addition to determination of GSSs, more than 130 trap mouse lines have been established and deposited to the CARD R-BASE; the database for cryopreserved embryos.

P91**THE MASK MUTATION REVEALS THE FUNCTION OF TRANSMEMBRANE SERINE PROTEASE 6 (TMPRSS6), WHICH REGULATES HEPCIDIN AND CONTROLS ABSORPTION OF DIETARY IRON**

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Here we describe *Mask*, a recessive chemically induced phenotype in mice that causes progressive loss of body (but not facial) hair. Regional alopecia results from severe iron deficiency, manifested by low serum iron and tissue iron stores, and microcytic anemia, all of which can be corrected by treatment with oral or parenteral iron. Upon repletion of iron, body hair growth normalizes. Iron deficiency in *Mask* mice is caused by reduced absorption of iron from the gastrointestinal tract. *Mask* mice express inappropriately high levels of hepcidin, a central regulator of iron homeostasis that suppresses gastrointestinal iron absorption and limits iron efflux from cellular stores. The *Mask* phenotype is caused by a splicing error in the gene encoding transmembrane serine protease 6 (*Tmprss6*), a gene that has not previously been targeted, and to which no function has previously been assigned. TMPRSS6 expression eliminates hemojuvelin-stimulated activation of the hepcidin promoter, and we have concluded that TMPRSS6 is a nonredundant component of the iron-regulated hepcidin suppression pathway. In one possible scenario, TMPRSS6 may directly cleave hemojuvelin, either destroying it or liberating it from the cell surface, and thereby causing downregulation of hepcidin gene expression. In *Mask* mice, hepcidin production is inappropriately high despite a signal imparted by iron deficiency, and prevents enteric iron absorption. TMPRSS6 has thus been identified as the most proximal known component of the key pathway that senses iron and regulates the iron absorption.

P92**A DOMINANT-NEGATIVE MUTATION IN GDF5 GENERATED BY ENU MUTAGENESIS IMPAIRS JOINT FORMATION AND CAUSES OSTEOARTHRITIS (OA): A NOVEL MOUSE MODEL FOR OA**

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GDF5 (Growth and differentiation factor 5) belongs to a subgroup of the TGF-beta/BMP family. GDF5 has been implicated in chondrogenesis and joint formation; however, its *in vivo* function remains mostly unclarified. While various human GDF5 mutations and their phenotypic consequences have been described, only loss-of-function mutations that cause brachypodism (shortening of the digits) have been reported in mice. Here, we report a new *Gdf5* allele obtained through a large-scale ENU mutagenesis screen. This allele carries an amino-acid substitution (W408R) in a highly conserved region of the signaling domain of the GDF5 protein. The mutation is semi-dominant, showing brachypodism and digit ankylosis (joint fusion) in heterozygotes, and much more severe brachypodism, ankylosis of the knee joints and carpal/tarsal bones, and early-onset osteoarthritis (OA) of the elbow joints in homozygotes. The mutant GDF5 had no abnormality in secretion and dimerization, but it inhibited the intra-cellular signaling of the wild-type GDF5 in a dominant-negative fashion. This dominant-negative effect seems to be resulted from hetero-dimerization of the mutant and wild-type GDF5s. Taken together with our recent finding that a functional single nucleotide polymorphism (SNP) in GDF5 is associated with hip and knee OA in human and decrease of GDF5 transcription is implicated in the pathogenesis of OA [Miyamoto et al. *Nat Genet* 2007], these results indicate a critical role of GDF5 in joint formation and the development of OA. This mouse should serve as a good model for OA.

P93**A NOVEL *PTGIS* GENE MUTATION SPONTANEOUSLY FOUND IN AN ICR CLOSED COLONY MOUSE**

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We have studied abnormal kidney which was observed in an ICR male mouse. In this paper, we report genetic studies which demonstrated a point mutation occurred in prostaglandin I₂ (prostacyclin) synthase (*Ptgis*) gene. An ICR male mouse (No.43) was backcrossed to four F1 females obtained crossing a DBA/2 female and the No.43 ICR male. In the offspring, some mice showed abnormal kidneys, such necrosis and fibrosis. Biochemical study revealed that urea nitrogen in plasma of the mutant was significantly higher than those of the normal mice. Linkage analysis using microsatellite makers revealed that the mutation was mapped on chromosome 2. A fine mapping performed using 138 Ham (our Lab code) microsatellite markers showed a significant linkage to *D2Ham131*(165,818,799 bp). *Ptgis* has been reported in this region and the knockout mice showing the same phenotypes as our mutant has been already reported. Sequence of ten exons demonstrated that the first 5' nucleotide of intron 7-8 was substituted from G to T. It leads to AG-TT which means disruption of AG-GT rule. As a result, transcription of exon 7 was followed by that of intron 7-8 consisting of 32 bases. This transcription error led to stop codon at codon 401 in the abnormal mice, but at codon 502 in the normal mice. Abnormal kidneys reported in this study were caused by a point mutation of *Ptgis* which leads to transcription error. Although the knockout mice of this gene have been already produced, our mutant is the first one generated by spontaneous mutation.

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G3 RECESSIVE SCREENS FOR LOCI CONTROLLING MOUSE DEVELOPMENTDebora Bogani¹, Pam Siggers¹, Nick Warr¹, Marie Cormack², Sian Polley² & Andy Greenfield¹¹Mammalian Genetics Unit, MRC Harwell, Didcot, Oxfordshire OX11 0RD, UK, ²Mary Lyon Centre, MRC Harwell, Didcot, Oxfordshire OX11 0RD, UK

We have established two three generation (G3) breeding schemes in order to identify recessive mutant alleles that cause developmental phenotypes. The use of distinct genetic backgrounds is anticipated to permit the identification of distinct phenotypic profiles. We have used N-ethyl-N-nitrosurea (ENU) to mutagenize C57BL/6J and BALB/C males (G0). Once these mice have recovered fertility they are bred to C3H/HeH and C57Bl/6J females, respectively. Males (G1) from these crosses are then used as founders of pedigrees generated by breeding with C3H/HeH and C57Bl/6J females. Females (G2) from these matings are backcrossed to their G1 fathers and G3 embryos are examined at 13.5, 14.5 or 17.5 dpc

Embryos are examined at 13.5/14.5 dpc for morphological abnormalities in a variety of organ systems, including neural tube, lung, heart, kidney and urogenital development. Left-right patterning and basic axis formation are also investigated. When specific pedigrees exhibit a higher than average number of dead/reabsorbed embryos, earlier time points are analyzed for phenotypes affecting basic body axis formation more likely to cause pre-mid-gestation lethality. In a subset of pedigrees fetuses are examined at 17.5 dpc for abnormalities of sexual development, including failure of testicular descent.

To date we have screened 130 pedigrees from the C3H/HeH X C57Bl/6J cross and 25 from the C57Bl/6JXBALB/C cross and recovered numerous mutants affecting left-right patterning, neural tube, limb, lung, heart, gonad development and early body patterning. Five mutations have been mapped, two cloned and one is at candidate gene sequencing stage. Details of a selection of these mutants will be presented.

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A NOVEL SPONTANEOUS STERILE MOUSE THAT WAS FOUND IN ICR CLOSED COLONY IS CAUSED BY *SMC1B* GENE MUTATION.Shuji Takabayashi¹, Yumika Yamauchi¹, Jiro Kimura¹, Motoko Noguchi² and Hideki Katoh¹¹Institute for Experimental Animals, Hamamatsu University School of Medicine, ²Department of Biological Science

Recently, we found that a male mouse of the ICR closed colony has a novel autosomal recessive gene causing sterility of both sexes. We describe here phenotypes of the sterile mice and the responsible gene.

Mice homozygous for the novel gene had normal weights and normal mating behavior in both sexes. However, they could not produce any offspring by mating with fertile mice. The sterile males had small testes at 1 month after birth. Histological studies using testes of the sterile males revealed that Sertoli cells and spermatogonia are observed, but spermatids and sperm are not observed in testicular seminiferous tubules. Females homozygous for this gene had oocytes which look normal, but they were sterile.

Gene mapping demonstrated that a gene responsible for the sterility is closely linked to *D15Mit235* (49.5cM) on Chr15. There were three candidate genes near the microsatellite marker. We focused on the structural maintenance of chromosomes 1B (*Smc1b*) gene among them and attempted to sequence the *Smc1b* genes of the mutant and normal mice. As a result, deletion of sixteen nucleotides was observed in exon 5 of the mutant gene. It was strongly suggested that the deletion led to a frame-shift generating a premature stop codon at position 775 (amino acid 247) in mRNA.

Revenkova et al. (2004) reported that the *Smc1b* gene knockout mice are sterile in both sexes. Our mutant mice showed almost the same phenotypes as those of the knockout mice. This is the first report of the *Smc1b* gene mutation occurred spontaneously.

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CHARACTERIZATION OF A NEW DWARFING MUTATION, PEEWEE, IN THE MOUSE

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PeeWee (PW) is a chemically induced recessive mutation in the Rump White region of chromosome 5. A PW line of the recessive mutation with a mixed C57BL/6J x 129 genetic background was established. The purpose of this study was to characterize the *PW* mutant and to determine the molecular and physiological basis for the dwarfing phenotype.

PW mice are normal without gaining weight slowly after weaning. *PW* mice are also fertile, but the number of mice per litter of *PW* mice was significantly lower than that of normal mice. All tissues examined including pituitary gland, brain and ovary appeared histologically normal. We analyzed plasma level of various factors and found that several growth factors altered in *PW* mice.

The *PW* mutation was mapped to chromosome 5 in an F2 intercross between *PW* mice and Balb/cJ mice. Fine structure mapping of the region showed that the *PW* locus is flanked by markers *D5Mit304* and *D5Mit356*, spanning a ~2 Mb region. The expression of 19 annotated within the critical region was analyzed by semi-quantitative RT-PCR analysis. *Slc10a4* expression was remarkably decreased in the brain and pituitary gland of *PW* mice. Sequencing the coding and genomic region of *Slc10a4* failed to reveal a mutation. This raises the possibilities that *PW* mutations resides in non-coding sequences, and regulate genes outside the genetically defined critical region.

In contrast to other mutations lead to dwarfing in mice, *PW* mice are relatively healthy. *PW* is a new model to investigate the regulation of body weight and size.

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SEARCHING FOR SOD1 ALS MODIFIERS IN THE MOUSE

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Amyotrophic Lateral Sclerosis (ALS) is a form of motor-neurone disease. It is a progressive, fatal, neurodegenerative disorder affecting both upper and lower motor-neurons. The majority of cases are sporadic, although familial cases are also encountered. SOD1 is the only major effect causative gene that has been found so far, accounting for less than 2% of the total cases. Transgenic mice carrying such mutations are a well establish model of the disease, as they resemble the patterns of degeneration seen on both familial and sporadic cases.

In order to try to find new genes specifically affecting the toxic gain-of-function of mutant SOD1 involved in ALS pathology, we have begun a modifier screen using N-ethyl-N-nitrosourea (ENU) random mutagenesis on the low copy number SOD1 G93A model. We injected BALB/c males (3 x 90mg/kg ENU), crossed them to G93A carrier females and assessed onset and progression of the disease in F1s using a variety of simple behavioural analyses and grip-strength measures. Here, we present the results of the baseline analysis for the low copy number SOD1 G93A, as well as the progress of the modifier screen.

P98**IDENTIFYING GENES THAT MODIFY HUNTINGTON'S DISEASE IN THE MOUSE**

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Huntington's disease (HD) is an autosomal dominant, progressive, fatal, neurodegenerative disorder caused by an expanded polyglutamine tract (PolyQ) in exon one of the Huntington's gene. Up to 70% of the variance in severity and age of onset is accounted for by the number of glutamine repeats. However, the rest of the variance is accounted for by environmental and genetic factors (modifiers). Genetic modifiers represent potential targets for the development of new therapeutic approaches to neurodegenerative disease. In order to identify modifiers modulating the HD phenotype, we have been pursuing a genetic screen using N-ethyl-N-nitrosourea (ENU) random mutagenesis on a mouse model of HD. We injected BALB/c males (2 x 100mg/kg ENU), crossed them to N171-82Q (HD mouse model) carrier females and assessed onset and progression of the disease in F1s using a variety of simple behavioural analyses and weight measures. Analysis of progeny to date has identified several mice lines carrying inherited dominant modifiers, including both suppressors and enhancers. One of the enhancers (*Guthrie*), affecting onset and severity of tremors, has been mapped to mouse chromosome 15. Others are currently being mapped, including a suppressor (*Panza*) delaying age-at-onset as well as greatly increasing survival.

P99**NMR BASED METABOLIC DISEASE MODEL MOUSE SCREENING IN ENU MUTAGENESIS PROGRAM**

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A large-scale mouse ENU (*N*-ethyl-*N*-nitrosourea) mutagenesis program in RIKEN consists of comprehensive variations of screening and analysis procedures. As a part of this program, NMR based screening was performed to isolate metabolic disease model mice. Firstly, urine and feces of 14 - 16 weeks male mice were collected using metabolic cage and analyzed by ¹H-NMR. Spectrums were compared between ENU mutagenized mice and non ENU treated same genetic background age-matched control mice. Mice that showed abnormal spectrums were then labeled by stable isotope for high sensitive detection of carbon containing metabolites (add ¹³C₆ -D-Glucose in drinking water). Urine and feces were collected again and ¹H-¹³C-NMR analysis was performed as a secondary analysis. As a result of this screening, 3 out of 96 mice showed different spectrums compared with control. From the result of secondary analysis by 2D-NMR, ethanol and malate were detected in urine from the three mice. Confirmation of the heritability is in progress. Organic acidemia is known to be a human disease where the organic acid is detected in urine. These mice are well like to be a model mouse of organic acidemia. From these results obtained, we considered that NMR based metabolome screening can be a useful means for searching metabolic disease model mouse. Emerging advantages of this method, i.e. comprehensive detectability of metabolites and its noninvasive character may encourage application to diagnosis of human diseases. Further examination is necessary for the development of high throughput metabolome screening toward the diagnosis of human metabolic disease.