CRISPR/Cas9 mediated mammalian genome editing

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It has been successfully demonstrated to edit target gene (knock-in of reporters or disruption of genes) by recently developed technologies, such as Zinc Finger Nucleases of artificial nuclease (ZFNs) or Transcription Activator-Like Effector Nucleases (TALENs). More recently, efficient genome editing has been reported by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system in variety of vertebrates as well as in mice, which would be more broadly used in the future.

In the animal facility of the Institute for Virus Research, Kyoto University, we are generating CRISPR/Cas9 mediated mutant mice by two different methods. First one is by Wang et al. reported in 2013, RNA is injected into the cytoplasm of mouse pronuclear stage fertilized eggs. Another one is by Ikawa et al. of Research Institute for Microbial Diseases, Osaka University, a plasmid is injected into the pronucleus. Although we observed no obvious difference in efficiency or reliability between these two methods at the moment, development of CRISPR/Cas9 system would be more important for genome editing such as generation of knock-in or knock-out mice. Here we would report a short summary of our experiments together with some examples of genome-edited mutant mice.

A new strategy for gene knock-in in Xenopus laevis using Platinum TALENs

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Although researchers are now able to easily generate gene knock-out animals using genome editing with TALENs and CRISPR/Cas9, existing strategy for gene knock-in is only applied to a limited species (e.g. mouse). Therefore, new targeting strategy for gene knock-in has been long wished in other animals. To solve this problem, we developed a new method for knock-in of donor vectors into target loci using Platinum TALENs*, designated as TAL-PITCh (TALEN-mediated Precise Integration into Target Chromosome). In this presentation, we would like to report experimental examples of gene knock-in in Xenopus laevis using TAL-PITCh and also appeal the feasibility of this strategy to other species.*Sakuma et al., Scientific Reports, 3: 3379, 2013.
O003-S  Induction of the single nucleotide mutation by CRISPR/Cas9 in mice
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The genome editing with engineered nucleases is an advanced technology for producing gene modified animals by directly injecting DNA or mRNA of site-specific engineered nucleases into the one-cell embryo. In genome editing, designation and construction of CRISPR/Cas9 system is easier than the others, moreover, when co-injection of CRISPR/Cas9 and single strand oligo DNA donor (ssDNA) is performed, it is possible to induce targeted small-scale gene mutation. The single nucleotide mutations (SNMs) are associated with a variety of human diseases. However, creation of SNMs-induced mice is difficult. In this study, we try to produce Albino C57BL/6 mice by CRISPR/Cas9 induced SNM in Tyr gene. The Tyr gene codes for enzyme tyrosinase that is necessary for melanine production. A couple of SNMs (291: G to T, 369: G to C) in Tyr are causes of Albino phenotype in mice. To induce the SNM G291T in Tyr, we injected CRISPR/Cas9 expression DNA vector and mutant ssDNA (G291T in Tyr) into 82 one-cell embryos from C57BL/6 mice. As result, 18 mice were obtained and 2 of which showed ocular albinism and absence of coat pigmentation. Genomic sequencing analysis revealed that the SNM, G291T in Tyr, occurred in one allele. We also found that other alleles were different deletion mutants at the target site. These results suggest that disease model animals could be generated by CRISPR/Cas9 mediated SNMs.

O004-S  Humanized CYP3A mouse and rat via chromosome engineering and genome editing technology
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Transgenic technology is useful for the analysis of phenotypic traits and genomic function. The Mb-sized genomic DNA could not be cloned into conventional cloning vector. To introduce regions of large human genes into cells or animals, we have developed a human and mouse artificial chromosome (HAC/MAC) vector. We have been generated humanized model mouse and rat expressing human drug metabolizing enzyme such as CYP3A, CYP2C and UGT2 cluster using the HAC/MAC vectors. CYP3A expression profile and the enzymatic function in human were well reproduced in the CYP3A humanized mouse, in which the endogenous mouse Cyp3a cluster was disrupted by Cre-loxP system. Rat CYP3A gene was knocked out via genome editing technology, TALEN, and CYP3A enzymatic activity in the rat liver microsome was suppressed. Next study is to generate the fully humanized CYP3A rat by mating the Cyp3a KO rat and CYP3A-MAC rat. Thus, chromosome engineering and genome editing technology will be useful for the generation of humanized animal model expressing drug metabolizing enzyme and disease model.
Highly stable maintenance of a mouse artificial chromosome in human cells and mice

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Human and mouse artificial chromosome (HAC/MAC) display several advantages as gene delivery vectors, such as stable episomal maintenance that avoids insertional mutations and the ability to carry large gene inserts including the regulatory elements. Previously, we showed that a MAC vector developed from a natural mouse chromosome by chromosome engineering was more stably maintained in adult mouse tissues than HAC vectors. In this study, to expand the utility for a gene delivery vector in human cells and mice, we investigated the long-term stability of the MACs in cultured human cells and transchromosomic mice. The MAC was stably maintained in human HT1080 cells in vitro during long-term culture. The MAC was stably maintained at least to the F8 and F4 generations in ICR and C57BL/6 backgrounds, respectively. Especially, the MAC was stably maintained in hematopoietic cells and tissues derived from old mice. Transchromosomic mice containing two or four copies of the MAC were generated by breeding. The DNA contents were comparable to the copy number of the MACs in each tissue examined, and the expression of the GFP gene on the MAC was dependent on the chromosomal copy number. Therefore, the MAC vector may be useful not only for gene delivery in mammalian cells but also for animal transgenesis.

Study for vitrification of rat preimplantation embryos

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Vitrification of embryos that can be preserved simply and rapidly has been developed in various animals. In rat, it was possible to cryopreserve stably in 2-cell embryos and later. Recently, use of pronuclear stage embryos is increasing for producing genetically engineered rats. This study was examined the development of the rat embryos that cryopreserved using vitrification methods. Pronuclear stage and 2-cell embryos were cryopreserved using vitrification solution (PEPeS) which contains propylene glycol, ethylene glycol, percoll and sucrose. Morphologically normal embryos were transferred into the oviducts of the pseudopregnant females. The development to offspring of pronuclear stage and 2-cell embryos were 14% and 33%, respectively. The development to 2-cell stage of embryos introduced or cryopreserved in PEPeS was 68% and 34%, respectively. Although rat pronuclear stage embryos could be cryopreserved, improvement of method is needed. These results also indicate that the cryopreservation of 2-cell embryos using this vitrification protocol can be applied for maintaining valuable rat strains as genetic resources.
O008-S  Chimeras created by injection of *Mus spretus* embryonic stem cells into tetraploid blastocysts

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**Purpose** *Mus spretus* is another species of mouse closely related to *Mus musculus* (common laboratory mice). However, there are few reports about *Mus spretus* embryonic stem cells. To investigate the totipotency of *Mus spretus* embryonic stem cells, we tried to produce chimeras by interspecific tetraploid complementation method.

**Methods** ES cells prepared from SPR2 (BRC) inbred strain were injected into tetraploid blastocysts stage embryos (MCH). The embryos were transferred into the uterus of recipient mouse (MCH).

**Results** Injection of *Mus spretus* ES cells into *Mus musculus* tetraploid blastocysts led to viable 100% chimeras of *Mus spretus*. This result supports the totipotency of *Mus spretus* embryonic stem cells and the utility of interspecific tetraploid complementation method between *Mus spretus* and *Mus musculus*.

O009-S  Effective generation of gene-manipulated rats from ES cells using frozen embryos

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Development of gene-manipulated rats derived from ES cells has long been expected, and recently establishment of ES cells derived from rat lines were developed. On the other hand, an effective generation method of gene-manipulated rats was not studied. Production of rat chimeric embryos, which requires a large number of blastocysts, but there is no stable technique established to obtain rat blastocysts. Thus, to improve the number and quality of embryos, we collected morula-stage embryos from the oviducts at 3.5 dpc and froze them with a simple vitrification method. These frozen molulae were cultured on mR1ECM for 24 hrs, and approx. 75% of them successfully developed to the blastocyst stage. The resultant blastocysts were injected with 4 different kinds of SD's ES cells and implantation was carried out. 47% of the transferred chimeric blastocysts were implanted to the uteri, and 50% of them developed into normal offspring. Out of these pups about 60% were chimera. In addition, the chimeric animals were able to transmit the ES cell-derived venus gene through the germ-line. These results show that cryopreserved rat molulae can be used for generation of gene-manipulated rats, and this technique help save time and costs required for the generation.
The role of large Maf transcription factors in the β-like cell conversion from mouse liver

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Recent studies have shown that MAFB and MAFA have their distinctive roles on β-cell development and maturation, respectively. However, it is not clear that the difference is due to the timing of gene expression, roughly Mafb before birth and Mafa after birth, or the specific gene function. Our aim is to examine the functional difference between these closely related genes of β-cells using an in vivo model of β-like cell production. We monitored the insulin transcriptional activity using bioluminescence emitted from the liver of insulin promoter-luciferase transgenic mice. Adenoviral gene transfers of Pdx1/Neurod/Mafa (PDA) and Pdx1/Neurod/Mafb (PDB) combinations generated intense luminescence from the liver with the peak emission 3 days after transduction and lasted more than a week. The peak signal intensities of PDA and PDB were comparable. However, PDA not PDB gene transfer resulted in significant luminescence on day 10, suggesting that Mafa has a more sustainable role on insulin gene activation than Mafb. PDA gene transfer induced several gene expressions necessary for glucose sensing and insulin secretion in the liver on day 9; however, glucose tolerance test and liver perfusion experiment did not show that induced β-like cells respond to high glucose concentration. These results suggest that Mafa has markedly intense and sustainable role on β-like cell production in comparison with Mafb.

Enhanced Engraftment of Human Hematopoietic Cells in Sheep After in Utero Transplantation

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We are generating human hematopoietic cells in vivo in sheep from human pluripotent stem cells. Although we have previously generated sheep having monkey hematopoietic cells after in utero transplantation (IUT) of monkey embryonic stem cells, the efficiency has been low. Here, we show improved engraftment of human (or monkey) hematopoietic cells in sheep using three modifications; a) pre-transplant treatment by the administration of busulfan to sheep fetuses (ie, the expansion of space for HSC engraftment in recipients), b) graft treatment by the HoxB4-transduction of HSCs (ie, the expansion of graft cells) and c) post-transplant treatment by the administration of stem-cell factor to the sheep (ie, selective expansion of engrafted cells).
Effective production of offspring from vitrified embryos in SPR2 (M.spretus) strain

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RIKEN BRC

Although wild-derived strains of mice often show poorer reproductive performance than laboratory mice, we reported successful results to obtain offspring from vitrified embryos in 33 wild-derived mouse strains belonging to *Mus musculus* (60th meeting). However, the preservation of other species in mice from the genus *Mus* such as SPR2 strain has been unsuccessful. The rates of females that ovulated after injection with 100 µl of anti-inhibin serum (AIS) or with 5 IU eCG followed by 2.5 IU hCG 48 h apart were 48% and 65%, respectively. The fertilization rate was very low (13%) irrespective of the method of superfertilization. When C57BL/6 oocytes were inseminated with SPR2 sperm, the fertilization rate was improved (67%). But the rate with the combination of SPR2 oocytes and C57BL/6 sperm was still low. Next, we tried to collect embryos by natural mating of SPR2 pairs after injection of females with AIS. In this method, a larger number of embryos (12 embryos per female) was obtained compared with IVF (2 embryos). A practically large number of offspring (55%) from vitrified-warmed embryos were produced by embryo transfer when pseudopregnant B6C3F1 females were used as recipients, while no offspring was born from ICR recipients. Thus, assisted reproductive techniques for SPR2 strain were successfully devised. We may expect that this strain can be efficiently used for studies of mouse genetics using interspecific genetic diversity.

In vitro fertilization with first-wave spermatozoa from immature male mice

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We have previously reported that normal offspring were produced by microinsemination with round spermatids of the first wave spermatogenesis from male mice at 17 days old (2004). Currently, mature males are used to produce offspring by natural mating or IVF using mature males. In this study, we examined whether first wave spermatozoa from immature males were usable for IVF. Spermatozoa were collected from the corpus and cauda epididymides of immature C57BL/6J males at 5 to 6 weeks old. IVF was then performed as follows: the sperm were preincubated in PVA-HTF containing 0.4 mM methyl-β-cyclodextrin for 1 h and added to drops of BSA-HTF supplemented with 1.25 mM GSH containing oocytes collected from mature females. The oocytes were examined for the presence of two pronuclei and sperm tail after staining with aceto-orcein. Sperm first appeared in the cauda epididymides at around 35 days of age, but their fertilizing ability in vitro was quite low. Three to five days later, the fertilization rates were dramatically increased. By contrast, spermatozoa in the corpus of epididymides showed a very low fertilizing ability in vitro, even with matured males. These results suggest that IVF can be successfully performed with first wave spermatozoa from premature males at 40 days after birth.
O015-S  Establishing the colony of a Metatherian species for a new experimental animal model  
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Mammals are divided into three subclasses which have living representatives; the Prototheria, Metatheria and Eutheria. While the mouse, which belongs to the Eutherian subclass, is most widely used in mammalian genetics and developmental biology, it is becoming increasingly clear that the mouse represents a specialized group among Eutherian mammals from various embryological and evolutionary standpoints. In order to understand the mammalian body formation more comprehensively, comparative studies using other Eutherian animals as well as Prototherian and Metatherian animals are essential. We have recently introduced a colony of a Metatherian species, opossum, which is similar to the mouse in body size and breeding characteristics.

Pairs of opossums were bred in the same breeding cages that were maintained at a temperature of 25°C and humidity of 60%. After two weeks, we separated the opossums and observed them to see whether they gave birth. Pregnancy rates, the number of pups and weaning rates were also satisfactory. These results suggest that Opossum is useful as new experimental animal.

O016-S  Development of a disease model of hemorrhagic fever with renal syndrome using mice  
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Hemorrhagic fever with renal syndrome (HFRS) caused by hantavirus infection is characterized by fever, renal dysfunction and hemorrhage. To understand the pathogenesis and develop therapeutic measures, animal model that mimics the human disease is necessary. Here we developed a disease model showing renal hemorrhage like in HFRS patients by using BALB/c mice. A clone of Korean hemorrhagic fever virus (KHFV cl-5) that was derived from a HFRS patient serum were obtained by plaque cloning and intravenously inoculated into 5–6 weeks old female BALB/c mice. KHFV cl-5 caused transient bodyweight loss in mice at 6–9 days post-inoculation (dpi) in a dose-dependent manner. Pathological examination demonstrated prominent hemorrhage of renal medulla in KHFV cl-5 infected mice at 9–12 dpi. Uric protein level was elevated at 6 dpi. Uric blood was detected from 6 dpi, peaked at 9 dpi and decreased at 12 dpi. Pretreatment of inoculum with immune serum of Hantaan virus that is belonged to the same serotype as KHFV cl-5 inhibited the manifestation of symptoms. Infected severe combined immunodeficiency mice with a defect of functional T and B cells showed continuous bodyweight loss and died without renal hemorrhage. In contrast, infected nude mice with a defect of T cells showed no symptom, suggesting that T cells have a role in developing renal hemorrhage. This is a first animal model of HFRS with renal hemorrhage.
**O017-S**  **Generation of novel mitochondrial disease model mice with mitochondrial tRNA gene mutation**

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Mitochondria possess multiple copies of mitochondrial DNA (mtDNA) as the unique genome, and mtDNA encodes the genes associated respiratory enzyme. The mutation of mtDNA have been known to cause mitochondrial disease, but its mechanisms and treatments have not been revealed yet owing to lack of technique generating transmitochondrial mice. Therefore, we conducted the study for the purpose of generation of mitochondrial disease mice with a mutation in the mtDNA tRNA<sub>Lys</sub> gene. The pathogenic mutation of tRNA<sub>Lys</sub> genes in mtDNA have been known to hotspot of myoclonic epilepsy with ragged-red fibers (MERRF). Therefore, we identified somatic mutations of tRNA<sub>Lys</sub> gene in mouse lung carcinoma P29 cells and one had G7731A mtDNA. Then, we transferred G7731A mtDNA into ES cells and obtained chimeric mice. Their mating with C57BL/6J male mice resulted in the generation of mice with G7731A mtDNA, named 'mito-mice-tRNA<sub>Lys</sub>7731'. At high proportions of G7731A mtDNA, mito-mice-tRNA<sub>Lys</sub>7731 expressed respiration defects and disease-related phenotypes and therefore are potential models for mitochondrial diseases due to mutations in the mitochondrial tRNA<sub>Lys</sub> gene.

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**O018-S**  **Characteristics of human tumors transplanted to a different immune-deficient mouse**

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The purpose of this study is to clarify the optimal type for heterotransplantation of human lung, colon or gastric cancer among the several immune-deficient mice, NOG (NOD/Shi-scid, IL-2Rγ null), SCID (C.B-17/lcr-scid/scidIcl) or nude (BALB/cAJcl-nu/nu). Six, 8-week-old male mice of each type were used in this experiment. Human tumor tissues, confirmed as cancer by pathologists, were collected from patients treated in the Fukushima Medical University hospital. The tumor tissues were cut into square-shaped pieces 1 sub-millimeter in thickness, and dipped in HBSS medium. Small slits were made in the right and left dorsal skin of each mouse, and 100 micro-L of the medium with tissue were injected subcutaneously. The heterotransplantation tumors were pathologically observed after the euthanasia. Though tissues of lung, colon, and gastric cancers showed successful heterotransplantation in the immune-deficient mice, the success ratio of heterotransplantation between each cancers and each types of mice were different. Lung cancer was successfully heterotransplanted only in one NOG mouse, conversely colon and gastric cancers showed successful heterotransplantation in all type of the immune-deficient mice. Pathological difference between heterotransplanted tumors in each immune-deficient mice was not observed in both colon and gastric cancers. In this study, NOG mice showed the highest success ratio of heterotransplantation for humanized animal model of cancers.
**O019-S**  An efficient implant method for developing an orthotopic lung cancer mouse model

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Inserting a needle into surgically exposed trachea has recently been reported as an implant method for developing an orthotopic lung cancer model in mice. However, this method may result in tumors forming in the neck as well as the lung. To develop an animal model in which the tumor forms consistently only in the lung, we devised a method of implanting cell suspensions of human lung cancer into the lung that did not need puncture operation. Referring to a previous report of an intratracheal implant method, we started by using an improved intubation stand and pharyngoscope and by designing the tracheal cannula used for implanting, etc. Then, we developed techniques by which cell suspensions could be injected into the lungs of mice with accuracy and verified using pigment liquid. Next, 5 cell lines (1x10⁶ cells/mouse) were implanted to SCID mice and the tumorigenesis rate in the lung was examined. Implantation by this method, which could be completed in about 2 minutes per mouse, resulted in injected pigment liquid being confirmed as present only in the lung in about 90% of mice, and the survival rate after injection was 100%. Secondly, although the tumorigenesis rate of one cell line was 0%, that in another cell line was 67%, and in three cell lines was 100%. From the above results, we think that the implant method considered here is useful for efficiently developing an orthotopic lung cancer mouse model.

**O020-S**  Validation of the ability of BALB/c substrains to hear high-frequency sounds

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We recently identified BALB/cA mice that exhibit high-frequency specific hearing loss (HFHL). In these mice, the threshold of auditory brainstem response (ABR) at the age of 1 month and age-dependent ABR threshold were both higher than those in C57BL/6J (B6) mice. Being an old strain, BALB/c has several extant and distantly related substrains that exhibit various phenotypes due to mutations or residual heterozygosity. To investigate whether the occurrence of HFHL was unique to BALB/cA, we assessed auditory perception in BALB/cA, BALB/cAn, BALB/cByJ, and BALB/cCr mice by measuring the ABR thresholds for tone-pip stimuli at 20, 24, 28, 32, and 36 kHz. ABR thresholds were measured at 1-month intervals for 1- to 5-month-old mice. At an ABR threshold range of 24-36 kHz, BALB/cA, cAn, and cCr mice exhibited earlier and more profound progressive hearing loss than cByJ mice. Moreover, at 3 months of age, the ABR thresholds of cA, cAn, and cCr at 32 and 36 kHz were 60 dB on average, which is greater than the normal thresholds of cByJ mice. Next, we performed whole-exome sequencing of BALB/cA and BALB/cByJ strains to screen for a candidate mutation associated with HFHL in BALB/cA mice. We detected genetic polymorphisms between cA and cByJ strains at 27,816 sites including 1,028 missense, 129 inframe-indels 127 frameshift, and 25 nonsense mutations.
**O021-S**  **Mechanisms underlying the dominant stereocilia phenotype are defined by Myo6 mutant analysis**
○Yuta Seki, Sari Suzuki, Yuki Miyasaka, Kunie Matsuoka, Yoshiaki Kikkawa

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Mutations in Myosin VI (MYO6) have been associated with autosomal recessive (DFNB37) and dominant (DFNA22) types of hearing loss in humans. Eleven Myo6 mutant mice have been reported. Homozygotes for these allele series exhibit profound deafness and circling behavior and have fused stereocilia in the inner ear hair cells. In contrast, most heterozygotes possessing both mutant and wild-type Myo6 alleles appear to have normal hearing and balance. In this study, we investigated hearing and balance in heterozygous mice carrying the novel Myo6 mutant, Myo6<sup>ksv</sup>, to assess its dominant phenotype. We analyzed the balance and hearing phenotypes by open-field behavior tests and measurements of the ABR. Although Myo6<sup>ksv/+</sup> mice exhibited normal vestibular function, their hearing ability was abnormal: the mice exhibited higher thresholds of ABR and rapid age-dependent elevation of ABR thresholds when compared with wild-type mice. In addition, we found disrupted and fused stereocilia in several hair cells in the cochlea of Myo6<sup>ksv/+</sup> mice, while western blot and immunohistochemical analysis showed reduced expression of the MYO protein in Myo6<sup>ksv/+</sup> mice. We also found mislocalized signals for MYO6 in the hair cells of Myo6<sup>ksv/+</sup> and Myo6<sup>ksv/ksv</sup> mice. These findings not only indicate the haploinsufficiency of MYO6, but also suggest that the dominant-negative effect of mutant MYO6 might be responsible for the expression of the dominant phenotype in Myo6<sup>ksv/+</sup> mice.

**O022-S**  **A novel mouse model for hearing impairment by selective ablation of OHCs in the inner ear**
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Mechanosensory outer hair cells (OHC) play an essential role in the amplification of sound-induced vibrations in the cochlea because of their ability to contract or elongate following changes in the intracellular potential. To study the critical roles of OHC, we generated a novel mouse model, OHC-TRECK, for hearing impairment by introducing the human diphtheria toxin (DT) receptor gene under the control of the OHC-specific promoter of the mouse prestin gene. DT administration to OHC-TRECK mice resulted in severe hearing impairment with a decrease in the amplitude of distortion product otoacoustic emissions and elevated auditory brainstem responses. Next, we assessed the phenotype for inner ear hair cells by immunohistochemistry using markers for hair cells, and observed that DT-administered OHC-TRECK mice exhibited specific depletion of OHC without any effects on inner hair cells and vestibular hair cells.
**O023-S**  
The analysis of the human chromosomal disorder, Partial trisomy distal 4q, using the model mouse

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Partial trisomy distal 4q (denoted 4q+) is a human chromosomal disorder caused by a duplication of the distal end of the long arm of chromosome 4 (Chr4). This disorder manifests typical phenotypes including growth and mental retardation, craniofacial, renal, heart, and thumb anomalies. These phenotypes likely result from an increased dosage of at least one gene located in the triplicated region. However, the responsible gene or genes and the molecular basis underlying the phenotypes remain unclear. Mouse models play crucial roles in understanding the gene-phenotype relationship in many human disorders. Previously, we found that spontaneous mouse mutant, recombination-induced mutation 4 (Rim4), is a model animal of 4q+. The Rim4 has an insertion of a 6.5-Mb fragment, which is syntenic to the distal end of human Chr4, 4q32.3 to 4q34.1 and shares typical phenotype of 4q+. Using this animal model, we disclose that gene dosage effect of some transcription factors is responsible for several phenotypes of 4q+. In addition to these results, we would like to report the mechanism of how 4q+ phenotypes are developed, and the excellent two methods to analyze the human chromosomal disorders: (1) high-resolution imaging of soft tissues such as model animal embryos using the contrast-enhanced µCT; (2) generation of new model animals of chromosomal disorders.

**O024-S**  
Generation of novel model of inflammatory diseases using IL-1-negative regulator genes knockout mice

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IL-1 is a proinflammatory cytokine and its bioactivity is negatively regulated by IL-1 receptor antagonist (Ra) and type 2 receptor (R2). IL-1Ra full knockout mice spontaneously develop various inflammatory diseases, such as rheumatoid arthritis and psoriasis-like dermatitis, thus they are thought as model mice of these diseases. IL-1Ra consists of some isoforms encoded by isoform-specific 1st exon and shared 3' exons. In this study, to generate novel disease model we newly developed isoform specific IL-1Ra knockout mice, and then these IL-1Ra and R2 double knockout mice were obtained by intercrossing these knockout mice. These mice were analyzed in both C57BL/6 and BALB/c backgrounds. In total IL-1Ra and R2 double knockout mice, a part of inflammatory diseases were deteriorated and novel disorders were developed. These phenotypes were segregated in isoform specific IL-1Ra and R2 double knockout mice. It is suggested that these mice are novel model of inflammatory diseases.
O025-S  Analysis of the molecular mechanisms which trigger autoinflammation in Ali18 mutant mice

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The Ali18 mutant mice was identified because of spontaneous inflammation on the peripheral limbs in ENU (N-ethyl-N-nitrosourea) mutagenesis. Although the Ali18 is thought to be a gain-of-function mutation in a tyrosine kinase, what kind of cell types induce autoinflammation in Ali18 mice is unknown. Since previous studies indicate that the bone marrow-derived innate immunity play a important role in the Ali18 phenotypes, we assumed mast cells as a candidate initiator of autoinflammation. In addition, histological analysis show that mast cell number was increased in inflammatory limbs of Ali18/Ali18 mice. W/W° mice, which is mutant alleles of c-kit gene, lack most of bone marrow-derived mast cells in whole body. Therefore, we tested whether double mutants with Ali18 and W/W° (Ali18; W/W°) show spontaneous inflammation without mast cells. Interestingly, none of Ali18/Ali18; W/W° showed spontaneous inflammation (0/13). In contrast, most of Ali18/Ali18; +/+ showed limb inflammation (5/6). We also analyze molecular properties of cultured mast cells derived from bone marrow of Ali18 mice. These results suggest that mast cells are initiator of autoinflammation in Ali18 mutant mice.

O026-S  ILC3s-derived IL-17 is involved in the pathogenesis of a novel Rag2−/−Il1rn−/− colitis model mice

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IL-1 receptor antagonist (IL-1Ra)-deficient (Il1rn−/−) mice spontaneously develop arthritis by an autoimmune mechanism. Here, we showed that Rag2−/−Il1rn−/− mice develop spontaneous colitis with high mortality, making a contrast to the arthritis suppression. Group 3 innate lymphoid cells (ILC3s) were expanded in the colon of Rag2−/− mice, and IL-17A production in ILC3s was increased in Rag2−/−Il1rn−/− mice. IL-17A-deficiency prolonged the survival of Rag2−/−Il1rn−/− mice, suggesting that IL-1-induced IL-17A from ILC3s play an important role in the pathogenicity of colonic inflammation. Although IL-17A-producing T cells were slightly increased in Il1rn−/− mice, these mice showed the expansion of CD4+Foxp3+ regulatory T (Treg) cells and no signs of colitic inflammation. On the other hand, Treg cells were absent in Rag2−/−Il1rn−/− mice, suggesting that Treg cells suppress the colonic inflammation by regulating IL-17-producing cells. These observations suggest that the balance between IL-17-producing cells and Treg cells is important to keep the immune homeostasis of the colon, and Rag2−/−Il1rn−/− mice are an useful colitis model for studying the pathogenic mechanism of colonic inflammation.
O027-S  CTRP3 plays an important role in the development of collagen-induced arthritis in mice
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Rheumatoid arthritis (RA) is an autoimmune disease that causes inflammation and bone destruction most commonly in joints. We previously generated two RA models, which spontaneously develop autoimmune arthritis. Because multiple genes are implicated in the development of autoimmune diseases, we searched for novel disease-related genes using DNA microarray techniques. The C1qtnf3 gene, which encodes CTRP3, is highly expressed in both models. In this study, we elucidated the pathogenic roles of CTRP3 in the development of arthritis. To elucidate the biological function of CTRP3, we generated C1qtnf3 KO mice. And we examined the collagen-induced arthritis in C1qtnf3 KO mice to assess the role of CTRP3 in the development of autoimmune arthritis. CTRP3 and complement C1q has a common domain (C1q domain), however, CTRP3-deficiency did not influence complement activation. Thus, CTRP3 is not complement regulator. We found that the incidence and severity score was higher in C1qtnf3 KO mice compared with wild-type mice. And also, the histopathology score was also more severe in C1qtnf3 KO mice. These observations indicate that CTRP3 plays an important role in the development of autoimmune arthritis, suggesting CTRP3 as a possible medicine to treat RA.

O028-S  Analysis of vertebral malformation induced by valproate via somite gene expression changes in mice
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In humans, many of congenital sporadic anomalies are believed to be due to environmental factors which influence gene expression. To elucidate gene expression changes by environmental cues, we employed valproic acid (VPA) induced vertebral malformation in mice as a model. VPA is a antiepileptic drug but also acts as teratogen to induce vertebral malformation of fetus. In addition, VPA is an inhibitor of histone deacetylase, thereby we assumed that epigenetic modification of gene expression leads to vertebral anomalies in this model. 500 mg/kg VPA was once administered intraperitoneally to ICR mice at the 9th day of pregnancy, then the mice were sacrificed to collected embryos. At embryonic day of development (e) 18, all embryos showed morphological vertebral abnormality such as hemivertebrae. Using e10 and e11 embryos, we analyzed gene expression patterns of Pax1 and Notch target genes by real-time qPCR. Interestingly, in e10 embryos, Pax1 expression levels were increased, and Tbx6 and Mesp2 expression levels were decreased. Furthermore, in situ hybridization (ISH) using Pax1, Tbx6, and Mesp2 probes revealed ectopic gene expression in somite borders and decreased gene expression in presomitic mesoderm. Our results may shed light on complex interrelationships between genetic and environmental factors which leads to sporadic vertebral malformation in humans.
O029-S  Search for biomarkers of type 2 diabetes using the SDT rat, a spontaneous animal model

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Spontaneous animal models of multifactorial diseases might be useful for searching biomarkers of the diseases. Metabolomics is a powerful tool to identify potential biomarkers. To investigate the usefulness of spontaneous models for searching biomarkers, we here performed metabolome analysis on longitudinal plasma samples of an animal model of non-obese type 2 diabetes, the spontaneously diabetic Torii (SDT) rat. The SDT (n=11) and control Sprague-Dawley (SD) (n=11) rats were phenotyped for body weight and blood glucose level from 6 to 25 weeks of age, and fasting plasma samples were collected longitudinally. Non-targeted metabolome analysis was performed on the plasma samples using gas chromatography-mass spectrometry (GC-MS). The SDT rats developed diabetes as early as 14 weeks of age, reaching 100% incidence by 20 weeks of age. At 12 weeks of age and later, several metabolites showed significant differences between SDT and SD rats. These metabolites represent potential biomarkers of the disease. Our data suggest that the longitudinal metabolome analysis on plasma samples of spontaneous animal models is useful for identification of biomarkers of multifactorial diseases.

O030-S  Establishment of a novel cystinosis rat model

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Cystinosis is a rare lysosomal storage disease characterized by the abnormal accumulation of cystine in various organs. Accumulation of cystine in all tissues eventually leads to multisystemic disease. The causative gene, CTNS, encodes cystinosin, the lysosomal cystine transporter. We reported a 13 bp deletion within exon 7 of Ctns in the LEA rat, and quantification of cystine in various tissues by HPLC. This study was conducted to establish a congenic strain carrying the mutant Ctns gene from LEA rat (from Ctns to d10rat80 1MB) on the F344 genetic background, to assume a pathologic condition as Cystinosis animal model. The levels of cystine in the tissues of congenic rat were significantly higher than those of control rat and markedly increased in spleen. In congenic rat, glucosuria were present in 100% at around 35 weeks of age. The histopathological analysis was performed to examine the renal lesions in 37 weeks of age. The disappearance of tubular epithelial cell layer associated with thickening of basement membrane was evident in proximal tubules. The Congenic rats lacking the function of Ctns is expected to be a useful for model animal of understanding cystinosis.
O031-S Characterization of renal retinoid X receptor in Han:SPRD Cy strain with polycystic kidney disease
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[Background and Aim]
Polycystic kidney disease (PKD) is the most common inherited renal disorder and is characterized by innumerable cysts. The mechanism of cyst formation is not clearly understood. We showed in a previous report that retinoid X receptor (RXR)-mediated signaling might be associated with cystogenesis in Han:SPRD Cy/Cy rats with PKD by DNA microarray. In current study, we demonstrated in detail the characteristic of renal RXR in PKD progression.

[Materials and Methods]
Whole protein was extracted from kidneys of 4-, 8- and 16-week-old Cy/+ or normal rats, respectively. Fractions of nuclear or cytoplasmic protein were extracted from kidneys of 16-week-old Cy/+ or normal rats, respectively. RXR was collected from renal whole protein by immunoprecipitation used RXR antibody. The expression, distribution, phosphorylation and its sites of RXR were detected with western blot analysis.

[Results and Discussion]
Expression levels of RXR in the Cy/+ kidney were increased dependent on age, indicating RXR is associated with progression of PKD. Most RXR was detected in the nuclear extract. Both Ser and Thr sites in RXR were phosphorylated, but not Tyr. It is known that phosphorylation of Ser and Thr sites in RXR induced aberrant cell proliferation in cancer cell. Therefore, these findings suggest phosphorylated RXR may relate to aberrant cell proliferation in PKD progression.

O032-S Characterization and genetic analysis of autosomal imperfect albinism in domestic chickens
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Imperfect albino chickens were found in a Fayoumi inbred line (GSP) at the Avian Bioscience Research Center of Nagoya University, Japan. The mutant is characterized by hypopigmentation of skin, feathers and eyes, resulting in colour dilution, and exhibits the defects of ocular morphology and function. The phenotypic trait of colour dilution is similar to the mouse pink-eyed dilution (p). To clarify the mode of inheritance and allelic association with tyrosinase-negative autosomal albino at the c locus, the mutant females were mated with males of the normal pigmented line (BM-C) and the c-locus albino line (CAL), respectively. The phenotypic segregation in F₁ and F₂ generations revealed that the imperfect albinism is controlled by a tyrosinase-independent autosomal recessive gene, for which the symbol p<sup>GSP</sup> is proposed. We isolated and sequenced a cDNA of the chicken p gene from the mutant, which is the causative gene for the mouse pink-eyed dilution, and identified a non-synonymous substitution from Ala (GCT) at position 370 to Asp (GAT) in the exon 11, which was specific to the p<sup>GSP</sup> allele. The Ala370 was located in the extracellular loop between the fifth and sixth transmembrane segments of the P protein, which was highly conserved in both avian and mammalian P proteins. Our present study provides useful information to elucidate the function of the p gene in pigmentation in a wide variety of animals.
O033-S  A novel missense mutation of Mip causes recessive congenital cataract in nat mice
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Mutations in the major intrinsic protein of lens fiber gene (Mip) produce an autosomal dominant cataract phenotype in humans, mice, and rats via either haploinsufficiency or dominant-negative effects. Here, we report a novel recessive cataract mouse model that carries a missense mutation in Mip. We generated backcross (N2) progeny between SJL-nat and BALB/cA mice. The segregation patterns of the phenotypes in the N2 progeny were consistent with the theoretical value for the autosomal recessive mode (P > 0.5). This result indicates that the gene for cataracts in nat mice is transmitted via autosomal recessive inheritance. We detected a point mutation in Mip that was a missense mutation leading to a glycine-to-arginine substitution (G631A). We investigated the MipG631A genotype in several inbred mouse strains. All of the tested strains except for nat exhibited a MipG631G genotype, and the affected glycine residue in MIP has been conserved in vertebrates. Although MIP is distributed throughout the plasma membrane of lens fiber cells in wild-type and heterozygous nat mice, mutant MIP is irregularly expressed in the nuclei of the lens fiber cells. Our data indicates that this missense mutation in Mip leads to a recessive cataract phenotype.

O034-S  A 1-bp insertion in Phldb1 causes rupture of lens cataract in rlc2 mice
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Rupture of lens cataract 2 (rlc2) is a mouse model carrying a recessive mutation that results in phenotypes such as disruption of lens capsule and subsequent rupture of lens at the posterior pole. The rlc2 mutation arose spontaneously in the KOR1 strain, and it was maintained in the BALB/cA congenic strain. To identify the mutation responsible for the rlc2 phenotype in mice, we performed whole exome sequencing (WES) in BALB/cA-rlc2 mice. We found a much higher mutation rate in a 40-70 Mb region on chromosome 9 than in other chromosomal regions of the reference sequence, which suggests that this region is derived from KOR1 mice. Sequence variants in this region were filtered against dbSNP and mouse resequence data to remove variants known to be present in other strains of mice. By WES analysis, we found a specific 1-bp insertion in Phldb1 gene of rlc2 mice, and this insertion was predicted to inactivate the PHLDB1 protein by a frameshift mutation which results in a truncated protein partially lacking the C-terminal PH domain. We found that PHLDB1 signals were suppressed in the epithelial cells of rlc2 mice, but PHLDB1 was specifically expressed in the lens epithelial cells and capsule. These results suggest that PHLDB1 plays an important role in the maintenance of the lens capsule.
O035-S  A dominant-negative mutation of *Psmb11* gene causes the severe T-lymphopenia in TN mice

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Thymus provides a specialized microenvironment, where distinct subsets of thymic epithelial cells (TECs) support T cell development. We have found a spontaneous mutant mouse line, which has an T-lymphopenia, in our in-house breeding colony of C57BL/6. These mice exhibited a significant reduction of naive T cells in peripheral blood. Compared with wild-type, TN (T-lymphopenia of naive population) mice had strikingly smaller size of thymus and number of thymocytes. The mating test indicated that T-lymphopenia was inherited as an autosomal dominant trait. By linkage analysis followed by deep-sequencing of the candidate region, we identified a missense mutation in the *Psmb11* gene from TN mice, which encodes cTEC-specific proteasome subunit β5t. To confirm the *Psm11* mutation was responsible for the TN phenotype, we performed CRISPR/Cas9-mediated genome editing in TN mice. Targeted disruption of *Psmb11* mutant allele in TN mice completely restored thymocyte cellularity up to wild-type levels, clearly indicating that *Psmb11* mutation is responsible for TN phenotype. Because well-characterized cTEC-deficient animal models have been limited, our findings prompted us to utilize TN mice for studying the significance of cTECs in T-cell development and immune system in vivo.

O036-S  Finding the loci associated with tame behavior using wild-derived heterogeneous stock mice

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Wild ancestors of domesticated animals had genetic variation for tameness. In mice, according to previous analyses for tame behavior in wild-derived strains (WS) and Laboratory strains, significantly behavioral differences were observed between these groups. In order to understand the genetic basis of tame behavior in mice, we were conducted genome-wide association studies (GWAS) using wild-derived heterogeneous stocks (WHS) which are originated from eight WS (MSM, HMI, BLG2, PGN2, KJR, CHD, NJL, and BFM/2). We obtained behavioral data related to tameness and 77K SNPs data from genotyping array (Mega MUGA) in 378 WHS mice. Using these data, we implemented the mixed linear model to map the loci associated with tame behavior. As a result, we identified several loci associated with two behavioral indexes (Heading, Locomotion) at genome-wide significance level. However, we could not find any causative genes that have registered around the candidate loci in MGI database. This result implies that there are novel genes or the novel function of known genes around the candidate loci. Here, we also show the ongoing project of artificial selection to detect the loci associated with tame behavior.
O037-S  Major QTL(s) on chromosome 10 for congenital hearing loss in NOD/Shi mice

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The NOD/Shi (NOD) mouse congenitally develops profound hearing loss due to stereocilia defects in the cochlear hair cells. The ahl2 locus that contributes to the hearing loss of NOD mice was previously mapped to chromosome 5 by linkage analysis of (NOD X C57BL/6J) X NOD backcross mice. Although we recently mapped QTLs on chromosome 5, including the ahl2 locus, using the same genetic cross, we found susceptible QTLs in other regions on the mouse chromosome. To define the major QTL, we performed genetic analysis of F2 mice using MSM/Ms (MSM) mice, which have good hearing ability in inbred mice. First, we assessed the auditory brainstem response (ABR) to tone-pip 4, 8, 16 and 32 kHz stimuli in F1 and F2 mice produced by crosses between NOD and MSM mice. The average ABR thresholds (37.4 dB) of the F1 mice tested at 1 month of age were statistically different from the ABR thresholds of NOD (96.4 dB) and MSM (23.9 dB) mice. The distribution of ABR thresholds to the stimuli of all tested frequencies in the F2 mice clearly showed a bell shaped normal distribution, suggesting that NOD mice develop hearing loss by a combination of one major and one minor QTL. Employing QTL analysis, we identified the major QTLs on a 30 Mb region of chromosome 10 that significantly affected the ABR threshold.

O038-S  Multiple genetic loci associated with ocular malformations in NAK/Nokh rats

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The developmental ocular disorder microphthalmia is one of the most profound congenital eye diseases and can lead to the complete loss of vision. Nodai aphakia (NAK) is a novel microphthalmos rat strain isolated by spontaneous mutation from a Sprague-Dawley. During eye development in NAK embryos, lens placodes and optic vesicles were observed at embryonic day (E) 12.5, and lens vesicles and the neural retina were observed at E15.5. However, the development of these tissues was obviously delayed compared to that in wild-type, and the structures were completely lost by E16.5. Furthermore, abnormal localization of γ-crystallin in the lens vesicle and a delay in PAX2 expression in the optic nerve were observed in NAK embryos. The results from TUNEL and BrdU assays indicate that NAK anophthalmia is associated with apoptosis and a reduction in cell proliferation. Although normal eye weight was observed in all the F1 progeny, the N2 and F2 progeny generated by backcrosses between NAK and BN or WI rats exhibited various phenotypes. By interval mapping using N2 progeny generated by backcrossing to BN and WI rats, a candidate locus with an LOD score greater than 3 was detected in a 62-71Mb region of chromosome 16. Therefore, we predict that microphthalmia in NAK rats is caused by mutations in several genes in this region.
O039-S  
**Pde6b^{rd1}** mutation is a modifier for early-onset cataract in Foxe3^{rc1} mice

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We previously used linkage analysis of an intercross between Foxe3^{rc1} and MSM mice to identify several modifier genes causing early cataractogenesis in Foxe3^{rc1} mice and showed that the rd1 mutation in Pde6b is the most promising candidate gene for the mrct locus. In this study, we performed phenotypic analyses of mice that were transgenic for a BAC clone (MSMg01-521B8) that covers the Pde6b region (Pde6b-BAC Tg mice) and mice that were congenic for Pde6b^{rd1}. Although SJL-Foxe3^{rc1} mice develop cataracts within 2 months of age, cataractogenesis in Pde6b-BAC Tg mice occurred at approximately 5 months of age. At 3 months of age, SJL-Foxe3^{rc1} mice showed profound lens opacity and large vacuoles in their lens fibers, but these phenotypes were milder in the Pde6b-BAC Tg mice. To obtain supporting evidence, congenic mice were generated by crossing SJL-Foxe3^{rc1} and B6 (Pde6b^{+/-}) or C3H (Pde6b^{rd1}) mice. Fifty-seven percent of Foxe3^{rc1} individuals with the C3H genetic background developed cataracts by 4 months of age, whereas those with the B6 genetic background did not exhibit cataractogenesis even when over 7-months old. Therefore, we suggest that Pde6b^{rd1} is a risk factor for early cataractogenesis. However, there was an obvious difference in the incidence of early-onset cataracts between SJL-Foxe3^{rc1} and C3H- or B6-Foxe3^{rc1} mice. Thus, an additional genetic factor might be associated with early cataractogenesis.

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O040-S  
**Identification of loci controlling tumor progression in a Japanese wild-derived mouse strain, MSM/Ms**

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In this study, we showed that MSM/Ms mice exhibit dominant resistance when crossed with susceptible FVB/N mice and subjected to the two-stage skin carcinogenesis protocol using DMBA/TPA. A series of F1 backcross mice were generated by crossing p53^{+/-} or p53^{--} F1 (FVB/N × MSM/Ms) males with FVB/N female mice. In recent study, we successfully mapped highly significant linkage for late stage papillomas (>6 mm) at 20 weeks after initiation was mapped at D4SNP14 (80-91 Mb) on chromosome 4. To narrow down the candidate region and to identify these corresponding genes, we generated congenic strain lines and subjected these mice to the two-stage skin carcinogenesis protocol using DMBA/TPA. As a result, we narrowed down the candidate region for tumor progression loci located at 63-97 Mb on chromosome 4. This locus on chromosome 4, which maps near the Cdkn2a/p19^{Arf} gene, was entirely p53-dependent. Our data showing the complete disappearance of the chromosome 4 modifier locus in p53^{--} mice suggest that p19^{Arf} may play a major role in the transition to large papillomas.
Genetic analyses using chromosome 11 congenic strains originating from A/J and SM/J mice

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SMXA RI strains and A/J-11SM×SM consomic strains were established from inbred A/J and SM/J mice and used for genetic analyses of complex traits. An earlier analysis using A/J-11SM mice identified QTLs on Chr. 11 for blood triglyceride (TG) levels and red blood cell (RBC) counts. We developed Chr. 11 congeneric strains from A/J-11SM mice and measured their blood TG levels and RBC counts to map QTLs for these traits. Three Chr. 11 congeneric strains that possessed the SM/J genome in the distal region of Chr. 11 had significantly higher blood TG levels than A/J mice. A QTL for blood TG levels was previously located to the distal region of Chr. 11 of SMXA RI and (SM/J×A/J)F2 mice. The SM/J allele contributed to increased blood TG levels in both analyses. This data supports the interpretation that the QTL for blood TG levels is located distally on Chr. 11. Mean RBC counts of Chr. 11 congeneric strains showed a wide and continuous variation. Congenic mapping indicated that several loci for RBC counts were present on Chr. 11. To search for candidate genes within these regions of Chr. 11, we performed exome sequencing analysis in SM/J and A/J mice and identified candidate genes with non-synonymous coding SNPs for blood TG levels and RBC counts.

Genetic analyses of the susceptibility for streptozotocin-induced diabetes in A/J mice

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Streptozotocin (STZ) is a well-known diabetogenic agent that exerts a cytotoxic effect on pancreatic B-cells. Inbred mouse strains differ in their susceptibilities to the diabeticogenic effect of STZ treatment; however, the genetic basis of this variation has not yet been fully elucidated. Our analysis using A/J, SM/J and A/J-11SM mice identified a locus on Chr. 11 that influenced sensitivity to STZ. Accordingly, we developed Chr. 11 congeneric strains from A/J-11SM mice and measured their susceptibilities to STZ. Congenic mapping indicated that a major locus for STZ susceptibility was located between D11Mit163 (27.7Mb) and D11Mit51 (36.4Mb) on Chr. 11. Exome sequencing analysis of SM/J and A/J mice identified non-synonymous coding SNPs at the locus for N-methylpurine-DNA glycosylase (Mpg; 32.2Mb), suggesting this is a candidate gene for STZ susceptibility.
O044-S  A modifier locus for colitis associated with the ABCB1A dysfunction is on chromosome 16 in mice
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Genetic alterations in the gene for ATP-binding cassette, sub-family B (MDR/TAP), member 1A (ABCB1A) determine susceptibility to colitis in mice and humans. However, even with the susceptible allele, the colitis incidence greatly differs depending on individual or genetic background of mice. Abcb1a knockout mice with an FBV genetic background (FVB.129P2-Abcb1a<sup>tm1Bor</sup>) spontaneously develop severe colitis. Meanwhile, SAMP1 mice with a spontaneous loss-of-function Abcb1a allele do not develop colitis. Thus, genetic factors other than loss of ABCB1A function is crucial in determining susceptibility to colitis under the ABCB1A dysfunction in mice. To elucidate the genetic basis for the strain difference in the susceptibility, (FVB x SAMP1)<sup>F<sub>2</sub></sup> mice were bred. Nineteen out of the 201 <sup>F<sub>2</sub></sup> mice homozygous for the mutant Abcb1a gene developed colitis. Genetic association study with marker loci revealed that all the 19 <sup>F<sub>2</sub></sup> mice with colitis were homozygous for marker loci on chromosome 16, implying the existence of a recessive gene, homozygosity of which was prerequisite for colitis development. These data will form the basis for positional cloning of the gene, which will hopefully lead to elucidation of the mechanisms involved in development of colitis.

O045-S  Verification of the congenital nephropathy resistant locus using tensin2 mutant congenic mice
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Introduction
The ICGN mouse is a chronic kidney disease (CKD) model that presents common symptoms and pathological changes associated with a variety of kidney diseases. Previously, we found that a deletion mutation of the tensin2 gene (<i>Tns2<sup>nph</sup></i>) leading to the proteinuria in ICGN mice. We have also shown that congenic strains carrying the <i>Tns2<sup>nph</sup></i> mutation on a C57BL/6J (B6) or 129X1/SvJ genetic background exhibit milder phenotypes than do ICGN mice, indicating the presence of several modifier genes controlling the disease phenotype. Recently, we performed QTL analysis using backcross progenies from susceptible ICGN and resistant B6 mice, and identified resistant loci for CKD progression on Chrs 2 and 13. The purpose of this study is to prove the existence of the CKD resistant locus on Chr 2, which linked to all tested parameters for CKD.

Materials and Methods
ICGN congenic mice introgressed QTL on Chr 2 from the B6 mouse were generated by the speed congenic strategy. The progression of CKD was evaluated by a blood test and kidney histology in 16-week-old mice.

Result
No deterioration in hemoglobin, BUN and tubulointerstitial fibrosis was observed in the congenic mice. The pathological changes in the glomeruli, such as expansion of the mesangial matrix were milder as compared with wild-type ICGN mice.
To examine the genetic basis of male subfertility, we analyzed its relationship to sperm morphology in B10.MOL-TEN1 mice which showed high frequency of sperm-head abnormality. Segregation analysis showed that the abnormal sperm phenotype in B10.MOL-TEN1 was inherited and was predictably controlled by at least three loci. Further quantitative trait loci analysis indicated three statistically significant loci, Sperm-head morphology 3, Sperm-head morphology 4 and Sperm-head morphology 5. The regions of these three loci mapped were 23.55-25.95 centimorgan on chromosome 1, 37.29-45.87 centimorgan on chromosome X, and 64.03-77.64 centimorgan on chromosome 6, respectively. A multiply interactions of these loci, and involvement of at least one additional locus was predicted. We also found that male fertility, sperm count, reproductive organ weight and testis histology of this strain was normal. The mapped region of Shm3 was found coincidentally identical to that of Shm1 of B10.M strain, which has shown both male sub-fertility and high frequency of sperm-head abnormality. Comparison between mutant strains, B10.MOL-TEN1 and B10.M, and normal inbred strains of mice were addressed. These findings indicate a complicated relationship between sperm morphology and male subfertility.

We previously found a new coat color mutant gene, Oca2<sup>pcas</sup> (oculocutaneous albinism II; pink-eyed dilution castaneous), derived from Indonesian wild Mus musculus castaneus. During development of a congenic strain carrying this gene on the C57BL/6J genetic background, we discovered a novel spontaneous mutant whose eyes and coat hair changed with age. Ordinary mice homozygous for Oca2<sup>pcas</sup> have pink eyes and grey coat hair, and this phenotype remains unchanged throughout life. In a previous annual meeting of JALAS, we reported the characteristics of this novel mutant phenotype. Here we carried out mating experiments to estimate the mode of inheritance of the novel mutant phenotype. We also performed genome-wide DNA methylation analysis using the next generation sequencer Illumina HiSeq and compared the degree of DNA methylation between novel and ordinary mutants. Mating experiments suggested that the novel mutant phenotype may be controlled by a small number of genetic loci but may be modified by epigenetic factors. Genome-wide DNA methylation analysis revealed that the novel mutant mouse had a highly methylated genomic region on chromosome 13 and low methylated regions on chromosomes 13 and X when compared to the methylation status of the ordinary mutant mouse. We will check the methylation status of these genomic regions by an independent method in the near future.
O048-S  HGF attenuates degeneration of Purkinje cells and Bergmann glia in a knockin mouse model of SCA7

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Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant disorder associated with cerebellar neurodegeneration caused by expansion of a CAG repeat in the ataxin-7 gene. HGF, a pleiotrophic growth factor, displays highly potent neurotrophic activities on cerebellar neurons. The present study was designed to elucidate the effect of HGF on cerebellar neurodegeneration in a knockin mouse model of SCA7 (SCA7-KI mouse). SCA7-KI mice were crossed with transgenic mice overexpressing HGF (HGF-Tg mice) to produce SCA7-KI/HGF-Tg mice that were used to examine the phenotypic differences following HGF overexpression. The Purkinje cellular degeneration is thought to occur via cell-autonomous and non-cell autonomous mechanisms mediated by a reduction of glutamate transporter levels in Bergmann glia. The Purkinje cellular degeneration and reduced expression of glutamate transporters in the cerebellum of SCA7-KI mice were attenuated in the SCA7-KI/HGF-Tg mice. Phenotypic impairments exhibited by SCA7-KI mice during rotarod tests were alleviated in SCA7-KI/HGF-Tg mice. The bifunctional nature of HGF on both Purkinje cells and Bergmann glia highlight the potential therapeutic utility of HGF for SCA7 and related disorders.

O049-S  Histological and biochemical analysis of deficient mice in β4GalTs responsible for LacCer synthesis

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We have previously reported that β-1,4-galactosyltransferase-5 (β4GalT-5) is an enzyme responsible for the biosynthesis of lactosylceramide (LacCer) and further revealed that β4GalT-5 is an important factor in early developmental stage (Nishie et al., 2010). In addition, a recent study has reported that β4GalT-6, another family gene in β4GalTs, also acts as enzyme for LacCer synthesis (Tokuda et al., 2013). In the 60th annual meeting of JALAS, we reported that 1) LacCer synthesis was reduced approximately half in each brain of brain-specific β4GalT-5-deficient mice and β4GalT-6 null mice, 2) behavioral phenotypes of each gene deficient mice were different in several kinds of behavioral paradigms and 3) mRNAs of MBP and PLP that is related to myelin organization were changed in the cerebral cortex and medulla oblongata of each gene deficient mice compared with wild-type mice. Based on these findings we attempted to generate β4GalT-5 and β4GalT-6-double deficient mice to reveal the roles of these genes in LacCer synthesis and behavioral phenotypes.
O050-S  Cochlear gap junction complex is degraded by Connexin26 mutation in hereditary deafness
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Hereditary deafness affects about 1 in 2000 children and GJB2 gene mutation is most frequent cause for this disease in the world. GJB2 encodes connexin26 (Cx26), a component in cochlear gap junction. In this study, we analyze macromolecular change of gap junction plaques with two different types of Cx26 mutation as major classification of clinical case, one is a model of dominant negative type, Cx26R75W+ and the other is conditional gene deficient mouse, Cx26f/fP0Cre as a model for insufficiency of gap junction protein. Gap junction composed mainly of Cx26 and Cx30 in wild type mice formed large planar gap junction plaques (GJP). In contrast, Cx26R75W+ and Cx26f/fP0Cre showed fragmented small round GJPs around the cell border. In Cx26f/fP0Cre, some of the cells with Cx26 expression due to their cellular mosaicism showed normal large GJP with Cx26 and Cx30 only at the cell junction site between two Cx26 positive cells. These indicate that bilateral Cx26 expressions from both adjacent cells are essential for the formation of the cochlear linear GJP, and it is not compensated by other cochlear Connexins such as Connexin30.

In the present study, we demonstrated a new molecular pathology in most common hereditary deafness with different types of Connexin26 mutations, and this machinery can be a new target for drug design of hereditary deafness. (Kamiya et al., Journal of Clinical Investigation, in press)

O051-S  Genetic diversity of sensitivity to anxiolytic drug in wild-derived heterogeneous stock mice
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[Background] Diazepam is mainly used for anxiolytic drug in human, but this drug is known to have undesirable adverse effects. Its anxiolytic effect and adverse effects show individual differences, therefore it is necessary to understand genetic basis underlying their effects. Anxiety-like behaviors and sensitivity to drugs are quantitative traits that can be affected by multiple genes. Wild-derived mouse inbred strains show high anxiety-like behaviors and they have high genetic diversity among strains. Thus, they and their hybridized populations can be very useful for identifying quantitative traits related to anxiety.

[Objective] We aimed to establish analysis method of anxiolytic effect of diazepam, and tried to examine strain differences in wild-derived inbred strains and wild-derived heterogeneous stock mice (WHS).

[Method] Eight wild-derived inbred strains (MSM, HMI, BLG/2, PGN/2, KJR, CHD, NJL and BFM/2) and WHS are injected with vehicle in a volume of 1mg/kg at first day and with diazepam in the same volume at next day 30min before performing open-field test each day.

[Result] We found clear strain differences of sensitivity to diazepam in wild-derived strains, suggesting genetic analysis using WHS is highly useful.
O052-S  Validation of DOHaD theory using mouse model (3): Gene expression and genomic methylation in brain
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The developmental origins of health and disease paradigm (DOHaD) is a concept that fetal environmental factors affect the adult phenotypes such as metabolic diseases, psychiatric disorders, and developmental disorders. We are conducting experimental plans to validate the DOHaD theory in the pathogenesis of psychiatric disorders and developmental disorders using mouse models. As shown at JALAS meeting in last year, we provided AIN93G as control diet, low-protein diet, and low-protein diet with folic-acid supplement to pre-pregnant and pregnant mice and examined the body weight of mother and offspring, clinical biochemistry of mother, gene expression in the brain and the liver of neonates. In the study, the offspring which were exposed to malnutrition in utero exhibited increased activity in the home cage, decreased contact to novel object, and decreased social investigation. In this JALAS meeting, we will report that the adult offspring of LP group and LP+FA group exhibited different pattern of mRNA expression and genomic methylation. In addition, we will mention about the functional annotations of genes which expression and methylation pattern had changed.

O053-S  Suppression of Dectin-1 signaling inhibits the development of mouse intestinal inflammation
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As a C-type lectin receptor family member and the receptor for β-glucan, Dectin-1 is known to protect the host against fungal infection. Beta-glucan can potentially promote the intestinal mucosal immunity, but the role of Dectin-1 in mucosal immune system is still largely unknown. To investigate the role of Dectin-1 in the development of inflammatory bowel disease (IBD), we administrated Dectin-1 deficient (Clec7a−/−) mice with dextran sulfate sodium salt (DSS) and found that Clec7a−/− mice were significantly resistant to DSS-induced acute colitis compared to wild-type (WT) mice. Sequence analysis of bacterial 16S-rRNA region revealed dramatic change of one Gram-positive bacteria, Lactobacilli, in the colon of Clec7a−/− mice, and transfer of this bacteria into germ-free WT mice gave resistant to DSS-induced colitis and up-regulation of Foxp3+ regulatory T cell differentiation. Pre-treatment with Dectin-1 antagonist ligand suppressed DSS-colitis. These observations suggest that Dectin-1 promotes colitis by regulating the balance of intestinal microbiota and consequently by regulating the adoptive immunity. Target of Dectin-1 signaling suggests a beneficial therapy to suppress IBD.
O054-S  Transmission of mouse hepatitis virus in mice

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We compared virus transmission among three strains of MHV. Five-weeks-old, female C57BL/6J (B6) mice were inoculated intranasally (in) with MHV-Y, MHV-NuU or MHV-JHM strain. Infected mice were bred with two naïve B6 mice in separate cages for four weeks. Anti-MHV-antibody was examined by MONILISA MHV. Two naïve mice bred with the mouse infected with MHV-Y in four cages were all seroconverted. One naïve mouse bred with the mouse infected with MHV-NuU in two cages was seroconverted, and two naïve mice bred with the mouse infected with MHV-NuU in three cages were not seroconverted. Two mice inoculated in with MHV-JHM and two naïve mice in two cages were not seroconverted. Two naïve habitant mice bred with the mouse infected with MHV-JHM in three cages were not seroconverted. These data suggest that MHV-Y has higher transmission ability than MHV-NuU or MHV-JHM (Wilcoxon rank-sum test). MHV-Y and MHV-NuU were detected from all the organs examined. However, MHV-JHM was not detected from gastrointestinal tract. We could not detect MHV in feces by conventional plaque assay irrespective of viral strains. The lack of viral replication in the gastrointestinal tract might be responsible for the low transmission ability of MHV-JHM.

O055-S  Analysis of myopathy in mice expressing measles virus nucleocapsid protein

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Measles virus (MV), a member of the Morbillivirus genus in the Paramyxoviridae family, forms inclusion bodies in infected tissues regardless of the presence of infectious virion. Although MV inclusion bodies are frequently observed in various tissues histopathologically after severe infection of MV, the function of them is still unclear. We attempted to address the function of MV inclusion bodies by generating transgenic mice expressing MV nucleocapsid protein (MV-N), the main component of MV inclusion body. The expression of MV-N was restricted to the muscle tissues of a part of transgenic mice. The mice expressing MV-N in their muscle tissues (MV-N mice) showed the wasting symptom and muscle degeneration. The morphological features of myopathy in MV-N mice were similar to those of autophagic vacuolar myopathies in human patients. Autophagy-associated proteins accumulated in degenerative myofibers of MV-N mice. These changes observed in MV-N mice strongly implied the contribution of MV-N to myopathy. The MV-N mice used in this analysis should be useful to study the function of measles virus inclusion bodies and the pathogenic mechanism of autophagic vacuolar myopathies.
O056-S  Efficacy of recombinant measles virus expressing avian influenza virus antigen in macaque model

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There are urgent needs for development of effective vaccines against highly pathogenic avian influenza virus (HPAIV). We previously established a macaque infection model of HPAIV using a wild water bird derived-HPAIV strain (A/Whooper swan/Hokkaido/1/2008, H5N1). In this study, we utilized this macaque infection model to examine protective efficacy of vaccine candidate for HPAIV. We generated recombinant measles virus (MV) expressing HPAIV haemagglutinin (HA) protein. Infection of the rescued recombinant virus (rMV-Ed-HA) resulted in HA expression in vitro. Inoculation of rMV-Ed-HA to cynomolgus monkeys induced production of anti-HA antibody. After monkeys were challenged with the HPAIV, the vaccinated monkeys were recovered earlier from influenza symptoms, shedding of the virus ceased earlier, and virus distribution in the lung was smaller than control monkeys. These results suggest that rMV-Ed-HA is a candidate of an effective vaccine against HPAIV infection to protect from HPAI severity.

O057-T  Normal vaginal flora in Japanese macaques (Macaca fuscata fuscata)

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Lactobacilli are the predominant microorganisms in the vaginal flora of human beings, and are known to play an important role in protecting them from genital infections. However, it is not clear which laboratory animals are suitable as experimental models for studying the role of lactobacilli in the vagina of human beings. Japanese macaque (Macaca fuscata) is the primate species taxonomically closest to human beings. Therefore, we undertook a quantitative study of the vaginal flora of Japanese macaques according to Mitsuoka’s procedure for analyzing intestinal flora. In the vagina of mature Japanese macaques, Bacteroidaceae, gram-positive anaerobic cocci (GPAC) and Streptococci were the most frequently isolated (100%) organisms. Numbers of Bacteroidaceae, GPAC and Streptococci were 10^{8.0}, 10^{8.4} and 10^{7.1} CFU/vagina, respectively. On the other hand, the prevalence of lactobacilli was 57% and the number of lactobacilli was 10^{6.2} CFU/vagina. These results suggest that lactobacilli are not predominant bacteria in the vagina of mature Japanese macaques.
O058-T  Inspection of the increase deterrent for bacteria of the slightly acid hypochlorous acid water
○Kawagoe, M., Shibata, Y., Obata, T., Ikeda, K., Sato, M., Toida, K., Nibe, H., Basaki, K., Ikeda, T., Matsuda, Y.
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Background: In our section, we use "autoclave sterilization tap water" for the water supply of a mouse breeding in SPF. This is to prevent the problem that microbial contamination and an alga derived from water get mixed with. However, the autoclave water has a fault that a microbe is easy to propagate because free available chlorine disappears by high pressure steam sterilization. Therefore, we rebuilt facilities in our section four years ago, and all the buildings laid pipes in "slightly acid hypochlorous acid water (slightly acidic water) generation device" at time. We introduced slightly acid water for washing (sterilizing) and the water supply use to mice and rats. The slightly acid water is slightly acid (pH 5.0-6.5), and there is much free available chlorine. We used highly-concentrated (50-60ppm) slightly acidic water for washing, but examined the water supply use to mice and rats using low-concentrated (10ppm) slightly acidic water. Purpose: As a result of having examined an increase suppressant effect for pseudomonas aeruginosa and hay bacillus of the slightly acidic water in vitro, we reported that we had a remarkable effect at highly-concentrated (50-60ppm) slightly acidic water. We examine an increase suppressant effect of the slightly acidic water about Escherichia coli by this experiment. Conclusions: With the highly-concentrated (50-60ppm) slightly acidic water, we were able to confirm an increase restraint (sterilization) effective thing for Escherichia coli.

O059-S  Biofilm Formation by CAR Bacillus
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The cilia-associated respiratory bacillus (CARB), an unclassified, extracellular, gram-negative filamentous bacterium, colonizes the ciliated respiratory epithelium of rodents and causes persistent respiratory diseases. Recently, we developed CARB single culture system and determined its full genome sequences. BLAST (nr) search of about 1,200 coding sequences (CDS) suggested that 45% of CDS were annotated to function-unknown or predicted protein genes. In order to study gene expression regarding pathogenesis, we attempted to find phenotype change of CARB in various conditions and discovered classic type biofilm formation in CARB single culture. [Materials and Methods] SMR strain of CARB isolated from rat was cultured in Vero E6 cell culture supernatants (IMDM/10% fetal bovine serum) at 37 °C in 5% CO₂/5% air. Morphological changes were monitored under phase contrast microscopy. Grown CARB was confirmed by PCR. [Results and Discussion] When cultured in low attachment flask, CARB divided and grew in planktonic and floating state. Then, some bacteria gathered to make aggregates, which were constituted of bacteria, fibers and mucus substances. About 10 days later, grown aggregates contacted each other and formed classic type biofilms. Biofilm formation is related to survival of bacteria. Using this system, we can monitor biofilm formation under microscope. This discovery is important to clarify pathogenesis of CARB.
O060-S  Study of eae-gene positive *E. coli* infection in Common Marmosets (*Callithrix jacchus*)

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Objective: To reveal the entero-pathogenicity of eae-gene positive Escherichia coli (EPEC) in Common Marmosets (marmosets).

Materials and Methods: We included six, 11-12month-old, male marmosets that were confirmed at a prior testing to be free of EPEC infection. Of these, 4 animals and 2 animals comprised the infectious (IG) and negative control groups (NC), respectively. We used the EPEC R81 bacterial strain, which was obtained from bloody stool samples from a marmoset. Experimental groups. Animals in the IG were inoculated with a 2-mL bacterial suspension in sterile saline (5x10⁸CFU/mL), and animals in the NC were inoculated with 2 mL of sterile saline.

Results and Discussion: In the IG, 2 animals out of 4 showed symptoms of minor depression on post inoculation day 1 (PID1); one animal experienced bloody stools on PID1, and 3 animals showed bloody stool on PID2. On PID3, the 2 animals that showed depressive symptoms were sacrificed; the necropsy revealed retention of loose stool and colonic mucosal petechia in both animals. Spontaneous recovery of bloody stool was observed in two animals on PID4 and PID7, respectively. On the PID14 necropsy conducted on two animals each in the IG and NC, gross anatomy did not reveal anything significant except for adenopathy of the ileocecal lymph nodes in the IG animals. These results suggest that EPEC is a causative agent of temporary hemorrhagic colitis of marmosets.

O061-S  Development of preservation solution for cold storage of mouse cauda epididymides

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Cold transport of cauda epididymides is a useful technique for the shipment of genetically engineered mice. However, the fertility of cryopreserved sperm retrieved from cold-stored epididymides dramatically decreases in a time-dependent manner. In this study, we developed a preservation solution ([(Lifor®; Lifeblood Medical Inc.) + sphingosine-1-phosphate (S1P)]) for the cold storage of mouse cauda epididymidimal sperm to maintain fertilizing ability for a few days. Although the fertility of cryopreserved epididymidimal sperm after cold storage in Lifor® decreased in a time-dependent manner, we were able to control the reduction in sperm fertility at 72 hours by adding S1P into Lifor®.

Meanwhile, we had epididymides preserved in the new preservation solution transported to our center from domestic and international laboratories under cold storage. We were able to successfully produce fertilized oocytes via IVF using the cryopreserved epididymidimal sperm transported to our center, and obtained live pups after embryo transfer.
O062-S  
In vitro fertilization between cryopreserved genetically engineered mouse sperm and cryopreserved unfertilized C57BL/6J mouse oocytes  
○Tomoko Umeno1,2, Kiyoko Fukumoto1,2, Yukie Haruguchi1,2, Tomoko Kondo1,2, Yumi Takeshita1,2, Yuko Nakamuta1,2, Mari Iwamoto1, Fumi Takahashi1, Eri Kohagura1, Shuji Tsuchiyama1, Toru Takeo1, Naomi Nakagata1  
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The use of cryopreserved oocytes is advantageous, as the oocytes are ready for use in IVF immediately after warming. In this study, we investigated the efficacy of IVF between cryopreserved genetically engineered mouse (GEM) sperm and cryopreserved unfertilized oocytes of C57BL/6 strain mice. We also evaluated the developmental ability of the embryos produced via the aforementioned IVF. In this investigation, we first prepared sperm from 5 different strains of genetically engineered mature male mice and C57BL/6J female mice oocytes (CLEA Japan, Tokyo, Japan) via our vitrification method. After thawing the gametes, we carried out IVF to evaluate fertility, and then transferred the embryos produced to recipient female mice to evaluate the developmental ability of the embryos. Results showed that the average rate of fertilization was 85%, while transferred 2-cell embryos normally developed to live pups (birth rate: 44%). These results suggest that cryopreserved oocytes are useful for producing pups via IVF using GEM cryopreserved sperm.

O063-S  
Fertilization and developmental ability of mouse oocytes derived from mature females  
○Yukie Haruguchi, Kiyoko Fukumoto, Tomoko Kondo, Yumi Takeshita, Yuko Nakamuta, Tomoko Umeno, Mari Iwamoto, Fumi Takahashi, Eri Kohagura, Shuji Tsuchiyama, Toru Takeo, Naomi Nakagata  
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Mature female mice over 1 year old are difficult to make pregnant through natural mating. However, there is not enough known about the reasons for infertility in mature female mice. In this study, we examined fertilization and developmental ability of mouse oocytes derived from mature females. Oocytes were collected from mature female mice of the C57BL/6J strain (20 to 60 weeks old) after superovulation. The collected oocytes were cryopreserved via our vitrification method. After warming the oocytes, the oocytes were used in in vitro fertilization (IVF) with freshly harvested sperm obtained from mature C57BL/6J male mice. The 2-cell embryos produced were transferred to pseudopregnant female mice of the ICR strain. Mature female mice showed decreased sensitivity to superovulation treatment (20 weeks old: 93.3%, 60 weeks old: 52.6%). Moreover, the number of ovulated oocytes decreased rapidly after 50 weeks of age (20 weeks old: 15.2, 60 weeks old: 4.9). However, the quality of vitrified-warmed oocytes taken from mature female mice was similar to that of the oocytes taken from young females. The mature oocytes showed high survivability after vitrifying and warming, high fertility in IVF and normal developmental ability to live pups after embryo transfer.
O064-S  **In vitro fertilization using frozen mouse sperm derived from cauda epididymis or vas deferens**

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Our protocols for sperm cryopreservation and **in vitro** fertilization (IVF) have been adopted to preserve genetically engineered mice (GEM) worldwide. In our method of sperm cryopreservation, sperm are collected from cauda epididymides. Meanwhile, there are many frozen samples derived from GEM that have been prepared according to older methods, using sperm collected from cauda epididymides and vasa deferentia. However, the fertility of cryopreserved sperm derived from vasa deferentia is unknown. In this study, we evaluated the fertility of cryopreserved sperm derived from cauda epididymides or vasa deferentia via IVF. Sperm were collected from the cauda epididymides and vasa deferentia of matured C57BL/6J male mice. The sperm were cryopreserved using FERTIUP® Sperm Cryoprotectant (Kyudo Co. Ltd., Japan). After thawing, the sperm were preincubated in FERTIUP® Preincubation Medium (Kyudo Co. Ltd., Japan). Thereafter, an aliquot of sperm suspension was transferred into CARD MEDIUM (Kyudo Co. Ltd., Japan) containing oocytes collected from matured C57BL/6J female mice. The fertility rates of the epididymal sperm were high and stable (94.3~100.0%), whereas those of the sperm derived from vasa deferentia were unstable (14.0~97.8%).

O065-S  **Evaluating the Potential Utility of Artificial Insemination to mutant Mouse Lines**

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In mice, IVF is well established, which allows assisted reproduction of strains that pose difficulties in colony maintenance by natural breeding. However, efficient IVF requires proficiency in various technical skills, including collection of unfertilized eggs and transfer to recipients. In addition, collection of eggs requires sacrificing females, which is associated with inherent risks of strain loss in case of unfavorable outcomes of IVF. In this report, we have explored potential utility of artificial insemination (AI) in the mouse that has been more regularly used in larger farm animals. In mice, routine use of AI has been hampered by somewhat inconsistent menstrual cycles that often complicate accurate determination of insemination timing. Recently, however, Ohwada et al. in Yamagata University have reported the BMY protocol which allows for more controlled ovulation cycles. Adopting this protocol, we have performed AI on CD-1 and C57BL/6J strains. In both cases, our attempts have yielded litters at high efficiencies. Currently, we are testing this method to one of mutant mouse lines that has shown poor performance in natural matings. Our preliminary tests on these mutant mice have resulted in successful pregnancy and yielded litters. These results suggest potential utility of AI in maintenance of strains that, in particular, have inherent problem in reproductive efficiency in natural mating set ups.
O066-S  In vitro fertilization between cryopreserved gametes in the MSM/Ms mouse strain

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MSM/Ms mice are very useful for comparative phenotypic analysis based on the difference in genetic polymorphism. However, an efficient system for producing MSM/Ms mice via reproductive technology has yet to be established due to the low number of ovulated oocytes and poor birth rate after embryo transfer. Oocyte cryopreservation helps us to prepare a sufficient number of oocytes collected from female mice for in vitro fertilization (IVF). In this study, we cryopreserved oocytes of MSM/Ms mice and examined their fertility and developmental ability. Unfertilized oocytes and epididymal spermatozoa taken from MSM/Ms mice were frozen separately and stored at -196°C in accordance with usual CARD methods (http://card.medic.kumamoto-u.ac.jp/card/japanese/kenkyu/sigen/manuals.html). After thawing, IVF was performed using these oocytes and spermatozoa. Embryos that developed to the two-cell stage via incubation in vitro were transferred to the oviducts of female recipients on the first day of pseudopregnancy (the day when a vaginal plug was confirmed). The rate of development to two-cell embryos was 86%. When these two-cell embryos were transferred to recipients, offspring were produced from 6% of the embryos.

O067-S  CARD-CNB Mouse Sperm and Embryo Cryopreservation Course in Spain

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Recently, the use of genetically engineered mice (GEM) has become prevalent for research in the field of life science. As a result, the need for mouse reproductive technology is skyrocketing in research institutes. The application of mouse reproductive technology in research projects using GEM dramatically improves the productivity of said projects. Up to now, we have provided a mouse bank service to facilitate research using GEM, and have developed various mouse reproductive techniques. In addition, we have held mouse reproductive technology workshops worldwide. Sharing this technology facilitates the transport and production of GEM, as it allows for the shipment of cryopreserved sperm or embryos and embryo transfer after IVF using the sperm. This in turn aids collaboration between domestic and international institutes. In the autumn of 2013, we held a successful international mouse reproductive technology workshop in CNB-CISC, Madrid. In this presentation, we will introduce the workshop and some of the findings obtained therein.
O068-S  **In vitro fertilization of transported cryopreserved genetically engineered mouse sperm**

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Cryopreserved sperm taken from those mice has been frequently transported between mouse banks and laboratories. However, there is no unified protocol for sperm cryopreservation amongst the mouse bank community. The variety of protocols is an obstacle hindering the efficient production of genetically engineered mice derived from transported cryopreserved sperm. This is proven by the low fertility rates for *in vitro* fertilization (IVF) at receiving facilities. To overcome this problem, in this study we developed a novel IVF protocol for cryopreserved sperm produced following different protocols in foreign institutes. Frozen-thawed mouse sperm transported from foreign institutes were centrifuged (300g, 5 minutes) and then incubated in FERTIUP\(^8\) mouse sperm preincubation medium (Kyudo Co. Ltd., Japan) for 30 minutes. Thereafter, oocytes preincubated in CARD MEDIUM (Kyudo Co. Ltd., Japan) for 60 minutes were incubated with preincubated sperm for 3 hours. After incubation, oocytes were washed and then cultured in HTF overnight. Fertilization rates for the frozen sperm transported from foreign institutes were 83.3~95.8%.

O069-T  **Usefulness of the UV-curing floor coating in the experimental animal facility**

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The floor of laboratory animal facilities is controlled at microbe level and is the factor that is important to clean degree maintenance. It is smooth, and dust particle removal efficiency by the cleaning is high, and the surface of the floor is not slippery, and, for chemical substances such as the disinfectant, it is required that it is the high durability. It is ideal to coat the floor surface to protect floor from pollution, a wound, the change in quality with chemical agents. However, the entrance of the builder to the management area is high-risk under management of the microbe control. Therefore the protection of the floor surface is difficult.

In this study, we examined wear-resistance, chemical-resistant and dust particle residual activity about usefulness of the UV-curing floor coating. Therefore, in non coating, wax coat, UV-curing coating, floor of this different surface treatment, we wiped off falling germs in breeding room floor after the cleaning and evaluated dust particle residual activity by culturing it.

Conclusion, UV-curing floor coating are the means that is effective to maintain laboratory animal facilities cleanliness environment.
O070-S  Vacuum-drying-process-less novel autoclave sterilization by the soft-hydrothermal processing

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We have developed a groundbreaking sterilization method by means of the "Soft-hydrothermal process", which has many advantages in terms of safety and cost-efficiency. This revolutionary technique makes it possible for sterilization to exclude vacuum-drying-process. In general, "Autoclaved sterilization" is used to sterilize cages and bedding before examine laboratory mice experimentation. But there is a critical concern which should be considered to prevent developing molds caused by condensed water vapor and sterilization failure. We have suggested that the "Soft-hydrothermal Processing" is sufficient to assure the complete dehydration reaction by sterilization of bath towel containing CI and BI, and the Sterilization Pack containing CI, BI, and a Bowie & Disk Test Pack. The cloth was completely dehydrated and CI, BI, and Bowie & Disk Test Pack were judged as complete sterilization meeting "Guideline for Sterility Assurance" by soft-hydrothermal processing for 121°C, 30min and/or 134°C, 4 min in the 100 % steam saturation ratio and in the flow system. These findings indicated that soft-hydrothermal processing, which was used in these conditions, has a strong effect of sterilization and might be applicable for dehydration, which does not requiring vacuum-drying-process, and retaining sterilization reliability and assurance.

O071-S  The effect of photocatalytic reactions on the degradation of waste inhalation anesthetics

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Recently, inhalation anesthesia has been popularized in the experiments of laboratory animals. Unnecessary exposure to waste anesthetic gases is undesirable in occupational safety. The purpose of this study was to investigate whether photocatalytic process effectively degrade trace anesthetic gases. One bag (control bag) was filled with vaporized anesthetic gases. The other bag (experimental bag) containing a photocatalyst filter was also with anesthetic gases. Both bags were placed in the darkroom. These bags were subsequently irradiated with long-wave ultraviolet lights (UVA). The changes in odor of anesthetic gases were chronologically determined via the odor sensor. In the control bag, neither light-shielding nor UVA-irradiation had effects on the odor sensor levels. After UVA-irradiation of the experimental bag, the odor sensor levels of anesthetic gases decreased linearly with the activities of photocatalysts. Our results showed that the changes of trace anesthetic gases were determined by the odor sensor. This study revealed that activated photocatalysts degraded trace anesthetic gases immediately after UVA irradiation. A charcoal canister should be used to remove the anesthetic agents from the waste gas stream. Additionally, in trace anesthetic gas scavenging systems, application of photocatalytic equipments is useful in maintaining occupational safety in laboratory animal facilities.
O072-T  Device of equipment for animal breeding

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We introduce our device and improvement about following breeding equipment for animal facility.

1. Separation panel for a mouse cage
   We partitioned a plastic mouse cage with use of a stainless-steel separation panel. Moreover, we developed auxiliary instrument to prevent mice from encroaching on the neighboring space when the cage was removed the lid. The result of this devise, we can use the breeding space effectively.

2. The ultrafiltration equipment for animal drinking water
   This equipment consists of an ultrafiltration water device, a chlorine addition device, control device and a water bottles filling device. Traditional ultra-filtration drinking water equipment needs installation work in animal facility. We devised that all devices making up the ultra-filtration drinking water equipment set in stainless-steel rack in a factory. The result of this device, we can eliminate noise and vibrations caused by installation work, and simplify procedure for purchase. Furthermore, this equipment reduces time of breeding work because it can fill 25 water bottles up with chlorine added ultra-filtration water in a short time. This equipment can connect to an automated watering system.

O073-T  Evaluation of bedding materials using radio telemetry system in rats

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The aim of this study was to investigate weights, body temperatures and activities of rats which bred with paper bedding material or wooden bedding material using a radio telemetry. Eight male CD(SD) rats (3 weeks old) were divided into two groups that were bred with paper bedding material (α-dry, Shepherd Specialty Papers; α-dry group, n=4) or with wooden bedding material (Tokojiki, Iwakura Co., Ltd.; wood chip group, n=4). Body temperature and activity of rats were measured using a radio telemetry (TA10TA-F10, DSI) every 10 minutes for 5 days at 3 weeks old, 7 weeks old, 11 weeks old and 15 weeks old. We measured body weights of the rats at same ages in weeks described above. In the both groups, it was observed light-dark cycle on the body temperatures and activities. The activity in the dark period was significantly higher in the wood chip group compared with the α-dry group at all ages. There was no significantly difference in the body weight, body temperature in light and dark periods and the activity in the light period between the wood chip group and the α-dry group. Kawakami et al. (Exp. Anim., 2012) reported that mice prefer the same color with their coat. CD(SD) rats shows the same tendency. It is possible that the rats bite and play with wood chips to relief for stress because the activities were different but body weights were not different between the two groups.
**O074-T**  
Education and Training Program of Animal Experiments of New employee in Kyowa Hakko Kirin Co., Ltd

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To improve the reliability of animal experimentation and promote experimental animal welfare, institutional animal care and use committee took the initiative in the organization (initial education, practical training, annual re-education, etc.), and the education and training of the animal workers in our company. In this paper we present the contents of the pre-educational training for new employees, and the point of education obtained from our experience.

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**O075-T**  
Emergency preparedness of Maruho Co. Ltd. labolatory animal facility

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In the experimental animal facility of Maruho Co. Ltd., even when the facility is damaged by accidents or disasters such as earthquakes, we decided to try to survive all animals. We have analyzed husbandry operations necessary for this purpose, and concluded that essential factors are: 1. worker, 2. water, 3. feed, 4. prevention of infection. We will report the preparation for these four factors.
O076-T  MHV Infection Accident and the Resolution in 2010
○Masato Kawaguchi1,2, Shigehisa Habe1, Naomi Shimizu1, Hirosi Nagashima2, Ryuichi Tajima2, Yuko Takayashiki2, Seiichi Tanaka1
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[Details] In the first microbial monitoring periodical inspection of 2010 in Center for Experimental Animals, Fukuoka University, two sentinel mice for microbial monitoring showed strong positive reaction to mouse hepatitis virus (MHV). All tests showed reproducibility. We requested Central Institute for Experimental Animals to confirmation test and the reaction proved positive. The result of the MHV test to all mouse rooms had been negative in the last microbial monitoring periodical inspection.

[Treatment] The rack supposed dirty area was set working guard line in last place and the rack was disinfected. After definite diagnosis, forbade entry to the applicable mouse room. Purchasing and receiving animals were stopped. We held the meeting for mouse users, 8 days later from the occurrence, and decided to dispose of 1486 mice by euthanasia. 29 days later from the occurrence, we confirmed that MHV prevalence was stopped and obtained the annulment of regulation. After that, mice of positive reaction to MHV haven't been discovered by periodical inspection.

[Discussion] We could prevent the spread of the infection within a short time. In hardware, we had set up safety racks with the use in mind of breeding mouse room. In software, our measures for infectious diseases gave the widest possible publicity to the animal care administration process and practice pathogenic microorganism monitoring inspection periodically. It is supposed that these things worked functionally for our succeed.

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O077-T  Tandem treatment of Ivermectin spray and pyrantel pamoate-medicated feed eliminated rat pinworms
○Toshiyuki Hayakawa, Hironobu Chiba, Chihiro Hino, Toshinori Samezawa, Daisuke Sasaya, Shigeru Inaba, Norihiko Shimizu, Wakana Shimada-Ooya, Hiroshi Funakoshi
Asahikawa Medical University Center Advanced Research and Education

Pinworm infection in rat is sometimes caused by transferred-infected animals from other animal institutions (even from abroad) and can be a common problem in University Animal Facilities. Pinworm eggs are resistant to various disinfectants and survive for a long time, and therefore, there is a risk of repeated infection. Medicated drinking water or food can be chosen as anthelmintic therapy. As the former treatment requires much care of changing the water and the latter requires less work and is easier to manage for technical staffs, medicated food is fundamentally useful for University Animal Facilities. However, delivery of medicated feed takes about a month and regulation of pinworms before the delivery is an important issue to be dissolved. Here we report that, rat pinworms:” S. muris that is classified in Category E" was efficiently eliminated from rats in our conventional rat animal room by pretreatment with Ivermectin spray (1mg/ cage once a week for 4 weeks) and subsequent treatment with pyrantel pamoate-medicated feed for 4 weeks in a tandem manner. In combination with washing racks and floor by hot water (60°C or above) and sterilization of hoses and nozzles by autoclave or ethylene oxide gas. Since end of our tandem treatment, there has been no repeated infection and no expansion sign to other rooms. Therefore, tandem treatment of Ivermectin spray and pyrantel pamoate-medicated feed is useful for treating rat pinworms in University Animal Institutions.
O078-T  A study of microbial monitoring test using the PCR method in NCGG -Contributions to 3Rs-

○Noboru Ogiso, Satomi Takano, Kaori Muguruma

Research Institute, National center for Geriatrics and Gerontology

The biological monitoring of rodents has been performed to confirm a quality of laboratory animals, or to clarify the infection status in facilities. In general, such traditional monitoring test use living animals, and has some troubles (such as a cost of test, animal transport). Recently, a new method for biological monitoring has been developed by Charles River Japan. This method needs skin swab, feces and urine of mice, and conducts PCR tests. Therefore, it will lead Replacement of mice. We now consider introduction of this method into our facility. Tests on both a SPF animal and an animal from the other research institute gave negative results in all inspection items (NCGG SPF level). Monitoring test with PCR is widely expected to contribute to three Rs (not only Replacement, but also Reduction and Refinement) at an animal facility. In the future, we plan to conduct monitoring tests for the same individual by a traditional and a new method. Furthermore, as there are some kinds of pathogenic organism which can be detect by PCR test for limited period, we may need to make a choice between two methods according to the purpose.

O079-T  Implementation system of microbial monitoring at Kanazawa University

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Animal facilities at Kanazawa University experienced extensive damages by outbreaks of infectious agents in 2008. External validation investigators also suggested the implementation of microbial monitoring. Following their suggestions, we established a standard system to monitor all facilities/sweets for mice and rats. In this presentation, we show the involvement of facility staff and the support by laboratory animal research facility.
Analysis of embryonic development and chromosomes of the chicken-quail intergeneric F\textsubscript{1} hybrid
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To investigate how lethality occurs in F\textsubscript{1} hybrids between chicken (2\textsubscript{n}=78) and Japanese quail (2\textsubscript{n}=78), we performed artificial insemination of chicken semen into quail hens. We incubated many eggs for 3-7 days and estimated the developmental stages of embryos. Fertility rate was 31\%, and 78\% of fertilized eggs showed developmental arrest by the 2-day stage, and 7\% of them contained dead embryos at the 2-3 day stage. The remaining 15\% contained viable embryos at the 3-day stage. Time course analysis revealed a developmental delay in hybrids, compared with quails: hybrid males hatched at 19-20 days, whereas quails hatched at 17-18 days. Many dead embryos exhibited morphological anomalies such as dwarfism and abnormal head shapes. Karyotype analysis of blastoderms and 3- and 7-day embryos revealed that the number of chromosomes of the hybrid was not different from 78. FISH analysis of metaphase spreads from hybrid embryos with chicken chromosome-specific DNAs and microchromosome-specific repetitive sequences cloned from both parental species indicated that chromosome complement of the hybrid consisted of a fifty-fifty mix of chicken and quail chromosomes. These results suggest that developmental defects such as growth retardation and abnormal morphogenesis frequently occur in hybrids, and mitotic chromosome nondisjunction rarely occurs in the hybrid.

Genetic differences between laboratory and wild Japanese quails
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\textsuperscript{1}Nagoya University, Nagoya, Japan \textsuperscript{2}Gifu University, Gifu, Japan \textsuperscript{3}Tokyo University of Agriculture, Tokyo, Japan \textsuperscript{4}National Institute for Environmental Studies, Ibaraki, Japan \textsuperscript{5}Nippon Institute for Biological Science, Tokyo, Japan \textsuperscript{6}NARO Institute of Livestock and Grassland Science, Ibaraki, Japan

The draft genome assembly of the Japanese quail (Coturnix japonica) enhances the importance of this species as an experimental animal model and provides clues to better understand genetic divergence in laboratory and wild quail populations. We assessed genetic differences between 15 laboratory lines and between laboratory lines and 16 wild quails using 50 microsatellite markers, which we developed based on the genome sequence data. Microsatellite markers revealed high levels of genetic differences not only among laboratory lines but also between laboratory and wild quail populations. Mitochondrial D-loop region exhibited three major haplotypes in laboratory lines, and two of the haplotypes were shared with wild quails. Three wild quails had unique haplotypes which branched off from basal nodes of the three major haplotypes described above in a phylogenetic tree. This result suggested different maternal origins of laboratory lines. Further genetic assessment would be needed to uncover a complicated breeding history of domestic quails, such as the geographical origin(s) and the global dispersal of domestic quails.
O082-S  Genome profiling of Slc:Wistar rats with SSLP markers
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¹ Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Kyoto, Japan ² EURATRANS, Kyoto University, Kyoto, Japan ³Osaka University of Pharmaceutical Sciences

Slc:Wistar outbred rats are widely used in biomedical research. A close similarity in the growth curve, survival rate, and immunological phenotypes was found between Slc:Wistar outbred and F344/NSlc inbred rats (Tayama et al, 1986). A genetic profiling of 9 disease-related variants also revealed a genetic similarity between them (Kuramoto et al, 2008). Here, we determined a genetic profile of the Slc:Wistar with 20 SSLP markers and compared it with that of F344 strains. DNAs were obtained from Slc:Wistar (n=31) and other available Wistar outbred rats; Crj:WI(Glx/BRL/Han)IGS (n=31), Crj:Wistar (n=31), Jcl:Wistar (n=32), BrlHan:WIST (n=32). Among 20 SSLP loci, 16 (80%) were fixed in the Slc:Wistar, 12 (60%) in the Jcl:Wistar, 4 (20%) in the BrlHan:WIST, Crj:WI(Glx/BRL/Han)IGS, or Crj:Wistar. Genetic profile of the Slc:Wistar that was made with the 16 fixed SSLP loci and 9 fixed disease-related loci was identical with those of F344/NSlc, F344/Jcl, and F344/Stm. These results indicated the low genetic variation of the Slc:Wistar and the close genetic similarity between the Slc:Wistar outbred and F344 inbred rats.

O083-S  Characterization of expression profiles in short isoform of the deafness gene Whirlin in mice
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The deafness gene Whirlin (Whrn) is conceptually translated into two isoforms of proteins, the long and short isoforms. Previous studies reported that both isoforms show different expression patterns during the developmental stages, distinct localization on stereocilia in the inner ear hair cells, and distinct phenotypes of stereocilia in deficient mice of both isoforms. In addition, multiple transcript variants were found in mouse and human tissues of each Whrn isoform. In this study, we performed molecular biological analysis to investigate the expression profile and transcriptional regulation of the short isoform.

We characterized the transcript variants of short-Whrn by RNA-seq and target RNA-seq analysis using a next generation sequencer. By comparing the depth of coverage among the different exons in short-Whrn, two isoforms that differ by an insert of exon 3 were detected in the mRNAs extracted from the cochlea, and their expression was confirmed by RT-PCR. Although alternative splicing was detected in the mRNAs, two isoforms were detected in eye mRNAs, and their transcripts were truncated for insertion of slight intron sequences. These results suggest that the transcription of two isoforms of short-Whrn was different among the various tissues. In addition, a previous study refined the regulatory region interval to a 13 kb region for short-Whrn expression. This study helps to describe the progress in determining the regulatory region using molecular biological analysis.
O084-S  

Meis1 is a crucial regulator of skin carcinogenesis

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Previous studies revealed Meis1 plays a significant role in blood development and vascular homeostasis and can function to help induce blood cancers, such as leukemia. However, to date, the role of Meis1 in epithelium remains largely unknown. Here we uncover two roles for Meis1 in the epidermis, we show that Meis1 functions as a critical regulator of a proto-oncogene factor in neoplastic tissue. In the normal epidermis, we show that Meis1 is predominantly expressed in the bulge region of the hair follicles where multipotent adult stem cells are maintained, and that the number of stem cells decreased in the conditional-epidermal Meis1 knockout mice after induction. In tumor tissue, we show that Meis1 expression increases as tumor development progresses from a benign to a malignant state. Furthermore, we show that mice lacking Meis1 in the epidermis have significantly lower number of benign and malignant tumors in the DMBA/TPA skin carcinogenesis mouse model revealing Meis1’s oncogenic role in tumor initiation and malignant conversion in skin tumorigenesis. Interestingly, we found that Meis1 localization was altered to neoplasia development. These findings suggest that during the transformation from normal to a hyperplastic tissue, a functional switch occurs in Meis1.

O085-S  

A nonsense mutation in the dystonin gene causes severe neuropathy in tdt mice

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We found a new recessive mutant mouse, torsion dystonia (tdt), in a colony of the JF1-Ednrb+/Ms (JF1-s+) strain that exhibits torsion behavior. Mouse homozygotes for the mutant allele are characterized by neuropathy, hypertonia, liberomotor defect, growth retardation defects and an early death in the postnatal stage. To identify the tdt mutation, we used a positional cloning approach. Linkage analysis using (C57BL/6J × JF1-s⁻tdt/+) F₂ mice restricted the potential tdt locus to the 1.5-Mb interval on chromosome 1. Sequence analysis of the genes located in this region revealed that the gene dystonin (Dst), which is a cytoskeletal linker protein whose loss of function in dystonia musculorum (dt) mice results in hereditary sensory neuropathy, carried a single point mutation leading to the substitution of a stop codon for glutamic acid at amino acid position 138. To examine the effect of the tdt mutation on Dst mRNA expression, we carried out real-time qRT-PCR using RNA from wild-type, tdt/+ heterozygous, and tdt/tdt homozygous mice. The relative abundance of Dst transcripts in the brains of tdt/+ and tdt/tdt mice was approximately 48 and 24% of wild-type levels, respectively. Thus, we hypothesize that this mutation leads to functional inactivation though the degradation of Dst mRNA by an mRNA decay mechanism.
HGF-overexpression suppresses gliosis & motoneuronal degeneration in a transgenic mouse model of ALS

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2Himeji Dokkyo University
3Korean University
4Osaka University Center for Advanced Science and Innovation

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive loss of brainstem and spinal motoneurons. Although prevention of motoneuronal degeneration has been postulated as the primary target for a cure, accumulating evidence suggests that microglial accumulation contributes to disease progression. This study was designed to assess the ability of HGF to modulate motoneuronal degeneration and microglial accumulation in the spinal and brainstem motor nuclei, using double transgenic mice overexpressing mutated SOD1(G93A) and HGF (G93A/HGF). Histological and immunohistochemical analyses of the tissues of G93A/HGF mice revealed a marked decrease in the number of microglia and reactive astrocytes and an attenuation of the loss of motoneurons in spinal, facial and hypoglossal nuclei compared with G93A mice. HGF overexpression attenuated monocyte chemoattractant protein-1 (MCP-1) induction, predominantly in astrocytes; suppressed activation of caspase-1, -3 and -9; and, increased X chromosome linked inhibition of apoptosis protein (XIAP) in the motoneurons of G93A mice. HGF reduces microglial accumulation by suppressing MCP-1 induction and prevents motoneuronal death through inhibition of pro-apoptotic protein activation. These findings suggest that, in addition to direct neurotrophic activity, HGF-suppression of gliosis may retard disease progression, making HGF a potential therapeutic agent for the treatment of ALS patients.

LOSS OF p62/SQSTM1 EXACERBATES MOTOR DYSFUNCTION IN ALS MOUSE MODELS

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Recent studies have revealed missense mutations in SQSTM1 in amyotrophic lateral sclerosis (ALS). SQSTM1 encodes p62/SQSTM1 that regulates the selective-autophagy via association with ubiquitinated proteins. Thus, p62 might play a crucial role in survival of motor neurons. However, the mechanism by which mutations in SQSTM1 cause ALS is still unclear. In this study, we investigate a role of p62 in the onset and/or progression of ALS using ALS mouse models. We generated SOD1H46R transgenic mice on a Sqstm1-null background. Body weight and survival of each animal were monitored. Motor coordination and balance were measured by a balance-beam test to evaluate the motor dysfunction. Sqstm1−/− mice did not show any gross abnormal phenotypes. By contrast, SOD1H46R and Sqstm1+/−;SOD1H46R mice both exhibited progressive motor dysfunction and paralysis with average life spans of 174 and 175 days, respectively. Remarkably, Sqstm1−/−;SOD1H46R mice showed a significantly-shorter life span of 154 days. Furthermore, a balance-beam test revealed that motor dysfunction in Sqstm1−/−;SOD1H46R mice occurs at an approximately 3 weeks earlier than that in SOD1H46R mice. These results indicate that lack of p62 exacerbates motor dysfunction in SOD1H46R mice.
O090-S  Database for retrieval of related disease information from phenotype data of mice and rats

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Mice and Rat play crucial roles as bio-resources of disease model applicable for experimental analyses. Data of functional analyses using these resources will be exponentially accumulated in the near future, and expected to contribute to disease and drug discovery studies. To meet these needs, the IT infrastructure to visualize their inter-relationships with diseases is desired.

We developed a database application to infer disease from phenotypic data in mouse and rat using ontologies. Phenotypic data were provided from three major phenotyping programs in Japan, Japan Mouse Clinic (RIKEN BRC), NBRP Rat Phenotyping Database (Kyoto Univ.) and NIG Mouse Phenotyping Database (Nat. Inst. Genet.). Phenotypic data is related to various biological ontologies such as species, anatomical parts of body and traits, by which users can filter phenotypic data. Phenotypic data are also related to the disease information via the Medical Ontology is provided from Tokyo Univ. Using this database, user can explore how similar phenotype mutant animals show to diseases without medical knowledge.

O091-T  Examination of the anesthetic protocol of a pig

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Induction of anesthesia in pigs is currently performed by the intramuscular administration, but the amount of drugs administered is large and distressing to the animal. In order to minimize the distress to the animal, we have examined the administration protocol. As a result, we were able to sedate the animals by administering one third of the amount of drugs in a slightly longer period.
O092-T Selection of the anesthetic drug replaced with Pentobarbitone

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Experiments were conducted by mixing the sedative and analgesic agents to examine alternatives to obtain the same effect as pentobarbital which is commonly used as an anesthetic in animal testing. We measured the time of arousal reaction and reflection after intraperitoneal administration of mixed agents to mice by providing a stimulus to the limb end, and summarized the results and discussed.

O093-T The influence by difference of the treatment time was confirmed in one rat AV shunt model

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We examined the influence of heparin-treatment time using a rat AV shunt model, and whether thrombus weight can be evaluated repeatedly throughout the experiment in the same animal. As a result, we were able to evaluate the treatment time, and suggest that measuring the amount of thrombus over the time using the same animals is an example of Reduction of 3Rs.
O094-S Establishment of immunosuppressive medication in microminipigs in transplantation study

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In vivo transplantation study using large animals can be of great value. Such a test has to include appropriate immunosuppressive (IS) medication. Microminipigs (mMP) have been emerged as a possible experimental animal model for transplantation study. Because the body size of mMP is smaller than general minipigs, mMPs have many merits of enabling easy handling and pharmacokinetic study using small amount of drugs. However, there is almost no report of IS-drug monitoring in mMPs. The aim of the present study is to design an IS protocol for mMPs. Two experiments were performed: (1) mMPs were received tacrolimus (p.o. or i.m.) once daily for 5 days. At day 5, changes of tacrolimus concentrations in blood were measured for 24 hours. (2) mMPs were received single dose of tacrolimus (p.o. or i.v.) and tacrolimus concentrations in blood were measured for 24 hours. As a result of experiment (1), 24-h blood trough level and AUC24h of tacrolimus were increased dose-dependently. It was found from experiment (2) that the bioavailability of tacrolimus by oral administration was lower in mMPs than in humans. These results showed that IS protocol for mMPs may need more amount of tacrolimus than for humans. Moreover, all animals seemed to tolerate the tacrolimus administration well.

O095-S Relationship between dental caries and dysfunctional salivary gland in alloxan-induced diabetic mice

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Alloxan (AL) treatment immediately induces hyperglycemia, and causes rapid-onset and progressive dental caries in rats. Furthermore, diabetes is related closely to dysfunctional salivary secretion in human. The aim of this study was to clarify the relationship between dental caries development and salivary function using AL-induced diabetic mice. At 1 and 3 month after AL treatment (75mg/kg, i.v.), the saliva volume was measured by stimulation of pilocarpine (PL) in half of mice, and all AL treated mice (DM) were subjected to histopathological examination for salivary glands in comparison with age-matched untreated mice (control). Severe hyperglycemia persisted, and the partial coronal defect was detected in a few cases of DM group, and the incidence tended to increase with age. Saliva volume in the DM group was significantly decreased. Histopathologically, decreased number of secretory granule and contraction of acinus, which are suspected to be due to stimulation of PL, were observed in the parotid, submandibular gland and lingual gland in PL-treated control group. However, these findings were not detected in PL-treated DM group at 3 months after AL treatment. On the other hand, there is no histological difference in salivary glands between both non-PL treated groups. Our results suggest that dysfunctional salivary secretion may become one of principal factors of dental caries in this model.
Development of Sexual Dimorphism in Mouse Pelvic Bone Patterning

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The mammalian pelvis is formed from the fusion of three endochondral bones (the ilium, ischium and pubis) into bilaterally paired innominate bones. This complex structure differs in both shape and size between males and females in nearly all mammalian species. The mechanisms for this sex difference in the pelvis are obscure, but they may include hormonal and genetic factors. To determine whether sex hormones can affect the sexual dimorphic development of the pelvis, we analyzed pelvis development in male and female mice. Histological analysis of pelvic bones of wild-type mice at 8 weeks of age has revealed that females have approximately twice the length of pubis and the cavity area of pelvis. This sex difference of pelvis was not evident until 3 weeks of age, suggesting the importance of sex hormones at the time of puberty. Gonadectomy of both sex enabled pelvic phenotypes intermediate type between male and female. Estrogen and androgen replacements led gonadectomized mice to female and male types of the pelvis respectively, regardless of the genotypic sex. These results indicate that the prototype of the pelvis is intermediate between males and females, and postpubertal androgen and estrogen induce male and female phenotypes, respectively. The progress we have made in analyzing phenotypes of mice deficient in estrogen and androgen receptors will also discussed.

Age-related hematological changes in male NOG mouse

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We have a collaborative program designed to derive and characterize mouse models, and to generate resources, information and innovative approaches to an application of the model animals for drug discovery and bio-maker. Generally, aging has significant effect on hematological parameters in mice and rat. The purpose of this study is to obtain hematological data from NOG (NOD/Shi-scid, IL2Rγnull) mouse. In this experiment, age-related hematological changes in NOG mouse (5 males) were compared to C57BL/6NJcl (B6; 3 males). At 6, 8, 12, 16, and 20 weeks of age, blood samples (0.05 mL) were collected from the facial vein of the animals under isoflurane anesthesia. Complete blood counts were measured with using an automated hematology analyzer (XT-1800iV, Sysmex Co., Ltd.). Moreover, lymphocyte, neutrophils, monocytes, eosinophils, and basophils were measured with the analyzer and observed on a blood smear by microscopy. The red cell counts in NOG and B6 mice increased with aging. In blood platelet counts, no change was observed associated with aging in both NOG and B6 mice. Compared with B6 mice, the white cell counts in NOG mice was low. Above all, the monocyte, neutrophil, and lymphocyte counts were increased significantly with aging. The present result is useful to decide on an effective timing for transplantation of cancer samples into the NOG mice.
O101-S  Examination for FOB in the common marmoset

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Purpose: The common marmoset (marmoset) is small primates, which their body weights are about the same as rats. Functional Observational Battery (FOB) is a test to evaluate the effect of a compound on the central nervous systems (CNS) in drug development. In this study, we investigated if the marmoset is useful to possess high-sensitivity primate-specific FOB.

Methods: Saline, Cocaine (Coc 2 or 1 mg/kg) or Chlorpromazine (Cpz 3 or 1 mg/kg) was intravenously administered to four male marmosets. FOB (general condition, motor function, etc.) was performed 5 minutes to 6 hours after the administration.

Results: Rapid and slight movements of the head which is specific for the non-human primate, continual movement, hyperirritability and hyperthermia were observed at 5 min after administration in Coc 2 mg/kg group. Although continual movement, hyperirritability and hyperthermia were also observed in Coc 1 mg/kg group, expression of other symptoms were decreased. Eye-closing, hypoactivity, slowed motion and ataxia were observed at 5 min after administration of Cpz 3 mg/kg. Eye-closing, hypoactivity were also observed in Cpz 1 mg/kg group, but expression of other symptoms were decreased.

Discussion: Dose dependent effects of Coc and Cpz on the CNS were able to be detected in the marmoset FOB. Since the primate-specific symptom such as rapid and slight of the head movement was observed in the Coc treatment, FOB in the marmosets may be more useful than that in rats.

1P001-T  Introduction of Institute of Laboratory Animal Research, Nagoya University

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In Higashiyama area of Nagoya University, Institute of Laboratory Animal Facility, a shared new SPF (specific pathogen free) facility, has opened. It is a three-story facility with total floor area of 2,218 m² for rats and mice. By this facility, we efficiently centralized the management of animals in Higashiyama area, and established a safer and more appropriate environment for animal experimentation.