Wednesday November, 5
10.45am – 12.15pm
Poster Session 3
Development/Cancer Biology/Evolution Aging; Mutagenesis; Neuroscience/Behavior; and Rat and Other Genomes
Posters P91- P123

S7/P91 – LINKING GENE EXPRESSION CHANGES TO PROTEIN SPATIAL LOCATION IN MUSCLE

Ashley Waardenberg[1], Toni Reverter[2], Christine Wells[1], Brian Dalrymple[2]
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P92/A DEFINING THE ROLE OF STRATIFIN AND I-KAPPA KINASE ALPHA IN EPIDERMAL DEVELOPMENT

Fang Liu[1], Gretchen Kusek[3], Melissa Behr[1], John Sundberg[4], Barbara Beyer[1], Bruce Herron[1]
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P92/B PARTIAL RESCUE OF THE ADRENOCORTICAL DYSPLASIA EMBRYONIC PHENOTYPE BY P53 DEFICIENCY

Christopher Vlangos, Bridget O'Connor, Madeleine Morley, Andrea Krause, Catherine Keegan
University of Michigan, Ann Arbor, MI, United States

S6/P93 - GENETICS AND BIOLOGY OF THE GERM CELL TUMOR SUSCEPTIBILITY LOCUS, *TER*.

Shirley Hammond, Amatul Ali, Sita Aggarwal, Chitralekha Bhattacharya, Kangli Luo, Angabin Matin
UT M.D. Anderson Cancer Center, Houston, Texas, United States

P94 IMPLICATION OF THE *RBPJ*, *NOTCHLESS* AND *STRAWBERRY NOTCH HOMOLOG 2* GENES IN THE CONTROL OF MELANOCYTE STEM CELLS HOMEOSTASIS

Genevieve Aubin-Houzelstein[1], Johanna Djian-Zaouche[1], Florence Bernex[2], Nelly Da Silva[3], Stephanie Gadin[2], Jean-Jacques Panthier[1]
[1]Institut Pasteur, Paris, France, [2]Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France, [3]Centre National de Référence en Hémobiologie Périnatale, Paris, France

P95 STUDYING SEXUAL DEVELOPMENT USING MUTAGENESIS IN THE MOUSE

Debora Bogani, Pamela Siggers, Rachel Brixey, Andy Greenfield
MRC Harwell, Didcot, United Kingdom

P96 MOLECULAR AND MOUSE LEVEL ANALYSES OF MULTIPLE POINT MUTANTIONS OF *BETA-CATENIN* GENE OBTAINED BY ENU-BASED GENE-DRIVEN MUTAGENESIS.

Takuya Murata[1], Norie Umemura[2], Emi Nakayama[2], Taichi Yamaguchi[2], Ai Nakahara[2], Kumiko Karouji[2], Yuichi Ishitsuka[1], Hayato Kotaki[1], Ryutaro Fukumura[1], Shigeru Makino[1], Yuji Nakai[1], Hideaki Toki[1], Hiromi Motegi[1], Hideki Kaneda[1], Tetsuo Noda[1], Shigeharu Wakana[1], Yoichi Gondo[1]
[1]RIKEN BRC, Tsukuba, Ibaraki, Japan, [2]RIKEN GSC, Yokohama, Kanagawa, Japan

P97 IDENTIFICATION OF TESTICULAR GERM CELL TUMOR SUSCEPTIBILITY GENES FROM THE 129. MOLF-CHR19 CONSOMIC STRAIN.

Rui Zhu, Shirley Hammond, Sara Ali, Angabin Matin
MD Anderson Cancer Center, Houston, TX, United States

P98 A NOVEL GENE AFFECTING CELL CYCLE PROGRESSION

Michael E. Rusiniak, William C. Burhans, Rosemary W. Elliott
Roswell Park Cancer Institute, Buffalo, NY, United States

P99 DOMINANT MUTATION OF *GSDMA3* IS INVOLVED IN MULTI-STEP SKIN TUMOR DEVELOPMENT

S Tanaka, M Tamura, T Shiroishi
National Institute of Genetics, Mishima, Japan

P100 ESSENTIAL ROLE OF *NANOS3* FOR ACQUISITION OF GERM CELL IDENTITY IN MICE

Satoru Kobayakawa[1], Yumiko Saga[2], Kuniya Abe[1]
[1]RIKEN BRC, Tsukuba, Japan, [2]National Institute of Genetics, Mishima, Japan

P101 THE MEDIATOR COMPLEX PROTEIN MED31 IS REQUIRED FOR MAMMALIAN DEVELOPMENT

Michael Risley, Kathryn Hentges
University of Manchester, Manchester, United Kingdom

P102 DEVELOPMENT OF A NOVEL ASSAY TO MAP ANGIOGENESIS QTLS IN PERINATAL MICE.

Fang Liu[2], Jason Smith[1], Richard Cole[1], Bruce Herron[1]
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P103 GENETIC BASIS OF NEURAL CREST DEFICIT IN DOWN SYNDROME MICE

Samantha Deitz, Jared Allen, Justin VanHorn, Randall Roper
Indiana University-Purdue University Indianapolis, Indianapolis, Indiana, United States

P104 MULTIDIRECTIONAL EFFECTS OF SINGLE – TRAIT, LONG TIME SELECTION OF MICE INBRED LINES.

Elzbieta Wirth - Dzieciolowska[1], Marta Gajewska[1], Dorota Lukasiewicz - Smietanska[2], Anna Kur[2]
[1]Oncology Centre and Institute, Warsaw, Poland, [2]Agricultural University, Warsaw, Poland

P105 *PRDM9*, A POSITIONAL CANDIDATE FOR THE MOUSE HYBRID STERILITY 1 GENE

Ondra Mihola[1], Zdenek Trachtulec[1], John C. Schimenti[2], Cestmir Vlcek[1], Jiri Forejt[1]
[1]Institute of Molecular Genetics AS CR, Prague, Czech Republic, [2]Cornell Univerity, Ithaca, NY, United States

S2/P106 – A NOVEL REGULATOR IN TERMINAL MEGAKARYOCYTE DIFFERENTIATION IDENTIFIED BY ENU MUTAGENESIS

Nicole Anderson[1], Zorana Berberovic[2], Esther Lau[1], William Stanford[1]
[1]University of Toronto, Toronto, On, Canada, [2]Toronto Center for Phenogenomics, Toronto, On, Canada

S1/P107 – IDENTIFICATION AND CHARACTERIZATION OF TWO MOUSE MUTANTS WITH DEVELOPMENTAL DEFECTS

Karen Mitchell, Kathryn Hentges
University of Manchester, Manchester, United Kingdom

S3/P108 - A SENSITIZED ENU MUTAGENESIS SCREEN FOR DOMINANT GENETIC MODIFIERS OF THROMBOSIS IN THE FACTOR V LEIDEN MOUSE

Randal Westrick, Sara Manning, Guojing Zhu, Catherine Lee-Mills, Jesse Plummer, David Ginsburg
University of Michigan, Ann Arbor, MI, United States

P109 MRC HARWELL – A CENTRE FOR GENE-DRIVEN MUTATION DETECTION

Mohamed Mohideen Quwailid, Zuzanna Tymowska-Lalanne, Anne Southwell, Debra Brooker, Sian Polley, Amanda Pickard, Martin Fray, Roger D. Cox, Paul Denny, Steve D.M. Brown
MRC Mammalian Genetics Unit, Harwell, Oxfordshire, United Kingdom

P110 A NONSENSE MUTATION IN THE MOUSE *ZDHHC13* GENE IS RESPONSIBLE FOR THE *LUCA* PHENOTYPE

Carlos Perez[1], Jean Jaubert[2], Isabelle Aubin[2], John DiGiovanni[1], Jean-Louis Guenet[2], Claudio Conti[1], Fernando Benavides[1]
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P111 WWW.FINDMICE.ORG -- FIND MICE, KNOCKOUTS, GENE TRAPS, MUTANT ES CELL LINES…

Janan Eppig, Mark Airey, Beverly Richards-Smith
The Jackson Laboratory, Bar Harbor, Maine, United States

P112 MICE GENOME STRESS IN THE CHERNOBYL SIMULATION EXPERIMENT

H Nakajima, T Saito, T Hongyo, T Todo
Osaka University, Osaka, Japan

P113 A RECESSIVE ENU SCREEN FOR NEW DEAFNESS LOCI

Rachel Hardisty-Hughes, Andrew Parker, Susan Morse, Gregory Ball, Sian Polley, Gemma Law, Susan Joyce, Emma Coghill, Stephen Brown
MRC Mammalian Genetics Unit, Harwell, Oxon, United Kingdom

P114 GENETIC AMALYSES OF INHERITED RETINAL DEGENERATION MODEL MOUSE IN ENU MUTAGENESIS

Tomohiro Suzuki[1], Hajime Sato[2], Kyoko Ikeda[1], Hiroshi Masuya[1], Haruka Yokoyama[1], Shizuka Nishimura[1], Hideki Kaneda[1], Ikuo Miura[1], Kimio Kobayashi[1], Hideaki Toki[1], Osamu Minowa[1], Yasuyuki Kurihara[3], Toshihiko Shiroishi[4], Shigeharu Wakana[1]
[1]Riken BRC, Tsukuba, Ibaraki, Japan, [2]Tohoku Univ., Sendai, Miyagi, Japan, [3]Yokohama National Univ., Yokohama, Kanagawa, Japan, [4]National Inst. of Genetics, Mishima, Shizuoka, Japan

P115 HIGH THROUGHPUT MOUSE KNOCK-OUT VECTOR DESIGN

Alejandro O. Mujica, Daniel Klose, Ruth Bennett, Vivek Iyer, Manousos Koutsourakis, William Skarnes
Wellcome Trust Sanger Institute, Hinxton-Cambridge, United Kingdom

P116 CHROMOSOME SUBSTITUTION STRAINS AND MOUSE MODELS OF DEPRESSION

Heena Lad[1], Cathy Fernandes[1], Hugo Oppelaar[2], Martien Kas[2], Leo Schalkwyk[1]
[1]Social, Genetic and Developmental Psychiatry, Institute of Psychiatry, Kings College London, London, United Kingdom, [2]Department of Neuroscience and Pharmacology, Rudolf Magnus Institute, University Medical Center, Utrecht, Netherlands

S12/P117 – MAPPING QTLS FOR MOUSE ANXIETY-RELATED BEHAVIOR USING CONSOMICS

Marijke C. Laarakker, Frauke Ohl, Hein A. van Lith
Department of Animals, Science & Society, Division of Laboratory Animal Science, Utrecht University & Rudolf Magnus Institute of Neuroscience, Utrecht, Netherlands

P118 REDUCING ANIMAL NUMBERS: SEQUENTIAL DESIGN OF BEHAVIORAL GENETIC EXPERIMENTS

Marijke C Laarakker, Frauke Ohl, Hein A van Lith
Department of Animals, Science & Society, Division of Laboratory Animal Science, Faculty of Veterinary Medicine & Rudolf Magnus Institute of Neuroscience, Utrecht University, Utrecht, Netherlands

P119 GENOTYPE × ENVIRONMENTAL INTERACTION IN SOCIAL DOMINANCE DETERMINATION

Alexander Osadchuk, Arkadiy Bragin, Evgeniy Brusentsev, Ludmila Osadchuk
Institute of Citology and Genetics, Novosibirsk, Russian Federation

P120 MUFFLED- A NEW MOUSE MODEL OF DEAFNESS

Richard Benjamin Gale, Rachel E Hardisty-Hughes, Andrew Parker, Emma Coghill, Sue Joyce, Steve D Brown
MRC Harwell, Harwell, United Kingdom

P121 COMPLEX GENETIC ARCHITECTURE OF SOCIAL INTERACTION AND AGGRESSIVE BEHAVIOR CLARIFIED USING CONSOMIC STRAINS DERIVED FROM MSM AND C57BL/6

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P122 DEMONSTRATING RESISTANCE-MITIGATING EFFECT OF *ARTEMISIA ANNUA* PHYTOCHEMICAL BLEND WITH *IN-VITRO* CULTURES OF *PLASMODIUM FALCIPARUM* AND *IN-VIVO* WITH *PLASMODIUM BERGHEI* ANKA IN MICE

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P123 HAVANA MANUAL ANNOTATION AND COMMUNITY INVOLVEMENT

Laurens Wilming[1], Denise Carvalho-Silva[1], Adam Frankish[1], Elizabeth Hart[1], Toby Hunt[1], Jane Loveland[1], Jonathan Mudge[1], James Reecy[2], Harminder Sehra[1], Catherine Snow[1], Charles Steward[1], Marie-Marthe Suner[1], Mark Thomas[1], Jennifer Harrow[1], Tim Hubbard[1]
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P92/A

DEFINING THE ROLE OF STRATIFIN AND I-KAPPA KINASE ALPHA IN EPIDERMAL DEVELOPMENT

Fang Liu[1], Gretchen Kusek[3], Melissa Behr[1], John Sundberg[4], Barbara Beyer[1], Bruce Herron[1]
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Four phenotypically similar mutations with defects in skin development have arisen in mice. Two mutations were mapped to distal Chr. 4: *pupoid fetus* (*pf*) and *repeated epilation* (*Er*). We have identified the Er mutation as a truncation in the 14-3-3 sigma protein (also known as stratifin (sfn)). The later two mutations were uncovered in gene targeting of factors that were previously characterized as inflammatory response genes: I-Kappa Kinase alpha (IKKα) and Interleukin regulatory factor 6 (Irf6).

Phenocopies represent a unique opportunity to discover how overlapping signal transduction networks can produce similar defects in development. In particular global expression analysis provides detailed information that enables distinction of similar phenotypes at the molecular level.

Here, we demonstrate that epidermis from $sfn^{er/er}$ and IKKα deficient mice share abnormalities in terminal differentiation, but IKKα epidermis has additional defects in keratinocyte proliferation. Expression analysis also shows that IKKα and Irf6 deficient skin have more similar expression profile than $sfn^{er/er}$ skin.

Our hypothesis is that Sfn, IKKα and Irf6 act through a common mechanism to maintain keratinocytes in an undifferentiated state while IKKα has additional effects on cellular proliferation. While no heritable mutations in sfn or IKKα have been reported in humans, stratifin expression is repressed by hyper-methylation of in many tumors. Recently, reports of IKKα mutations in aggressive squamous cell carcinomas have suggested additional biological links between these proteins.

Our ongoing work will identify important pathways that regulate proliferation/differentiation status of keratinocyte and determine their contribution to skin development and disease progression including skin cancer.

P92/B

PARTIAL RESCUE OF THE ADRENOCORTICAL DYSPLASIA EMBRYONIC PHENOTYPE BY P53 DEFICIENCY

Christopher Vlangos, Bridget O'Connor, Madeleine Morley, Andrea Krause, Catherine Keegan
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The autosomal recessive adrenocortical dysplasia (*acd*) mouse mutation arose spontaneously at the Jackson Laboratory. On the C57BL/6 background the embryonic *acd* phenotype includes caudal truncation, hydronephrosis, vertebral segmentation anomalies, limb defects, and perinatal lethality. The *Acd* gene encodes a telomeric protein (also known as Tpp1) that functions in a multiprotein complex to maintain telomere integrity. Based on the function of the *Acd* gene we hypothesized that the characteristic caudal truncation of *acd* mutant embryos is due to apoptosis or cell cycle arrest via activation of p53. Immunofluorescence experiments with the apoptosis marker caspase-3 demonstrated a significant increase in the number of apoptotic cells in *acd* embryos versus wildtype littermates. Measurement of proliferation using BrdU incorporation followed by immunofluorescence did not show any significant differences in *acd* mutant embryos. In order to determine whether the increased apoptosis we observed is p53 dependent, we crossed *acd* mice to p53 null mice and analyzed the phenotype of offspring from double heterozygous intercrosses. Although the embryonic lethality associated with the *acd* mutation on the C57BL/6 background was not rescued, the severe vertebral anomalies associated with the *acd* mutation were completely rescued in homozygous double mutant embryos. Furthermore, the limb hypoplasia previously observed in homozygous *acd* embryos was rescued by p53 haploinsufficiency, while complete p53 deficiency resulted in preaxial polydactyly, suggesting that p53 acts in a dose-dependent manner. These findings support the hypothesis that the skeletal anomalies of *acd* mice are secondary to p53-dependent apoptosis and demonstrate the importance of telomere maintenance during embryogenesis.

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P94
IMPLICATION OF THE *RBPJ*, *NOTCHLESS* AND *STRAWBERRY NOTCH HOMOLOG 2* GENES IN THE CONTROL OF MELANOCYTE STEM CELLS HOMEOSTASIS

Genevieve Aubin-Houzelstein[1], Johanna Djian-Zaouche[1], Florence Bernex[2], Nelly Da Silva[3], Stephanie Gadin[2], Jean-Jacques Panthier[1]
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To better understand the genetic control of melanocyte stem cells (MSCs) maintenance and biology, we chose to work on three coat color mutations: two conditional knock-out mutations, the first invalidating the *RbpJ* gene and the second the *Notchless* gene in the melanocyte lineage (respectively *Tg(Tyr-Cre)*; *RbpJ*$^{flox/flox}$ and *Tg(Tyr-Cre)*; *Nle*$^{flox/flox}$ mice, referred to as c*RbpJ* KO and c*Nle* KO for conditional *RbpJ* and *Nle* knock-outs); and a mutation leading to overexpression of the *Strawberry Notch homolog 2* gene in the melanocyte lineage (*Tg(Dct-Sbno2)*). We analyzed the corresponding coat color phenotypes at birth and during postnatal life. We show that although all three mutations affect the development of pigment cells, they act on different steps: the c*Nle* KO and the c*RbpJ* KO mutations affect melanoblasts survival at an early stage or during late embryogenesis respectively; the *Tg(Dct-Sbno2)* mutation affects melanoblasts migration. As the mutant mice got older, they all displayed coat color whitening. We studied the distribution of pigment cells on skin sections or dissected hair follicles at postnatal day 8 (P8) and P30. We found that in many hair follicles from c*RbpJ* KO and c*Nle* KO mice at P30, the MSCs and their progeny were in reduced number. In *Tg(Dct-Sbno2)* mice, many hair follicles at P30 had a reduced number of MSCs that gave no progeny at all. Our results suggest that the *RbpJ* and *Nle* genes control MSCs maintenance in a similar way, whereas *Sbno2* seems to be also involved in the differentiation of MSCs into transit amplifying melanoblasts.

P95
STUDYING SEXUAL DEVELOPMENT USING MUTAGENESIS IN THE MOUSE

Debora Bogani, Pamela Siggers, Rachel Brixey, Andy Greenfield
MRC Harwell, Didcot, United Kingdom

In mammals the gonadal sex of an individual is determined by the presence or absence of the Y chromosome and the subsequent activity of SRY during early male gonad development. In addition to SRY, a number of autosomal and X-linked gene products are also required for development of testes and ovaries in males and females, respectively. A number of lines of evidence suggest that our understanding of the sex determining pathway is far from complete. We have performed a forward genetic screen for loci controlling embryonic development in the mouse. ENU mutagenesis and a three generation (G3) breeding scheme have allowed the identification of recessive mutant alleles affecting a variety of developmental processes. One mutant pedigree identified included embryos exhibiting neural tube defects and abnormal male gonad development, with phenotypes ranging from disrupted testis cord formation to the presence of ovarian gonad morphology in XY individuals. We will describe the chromosomal mapping and molecular characterisation of the mutated locus and describe more detailed phenotypic characterisation of the mutant gonads on distinct genetic backgrounds.

P96

MOLECULAR AND MOUSE LEVEL ANALYSES OF MULTIPLE POINT MUTANTIONS OF *BETA-CATENIN* GENE OBTAINED BY ENU-BASED GENE-DRIVEN MUTAGENESIS.

Takuya Murata[1], Norie Umemura[2], Emi Nakayama[2], Taichi Yamaguchi[2], Ai Nakahara[2], Kumiko Karouji[2], Yuichi Ishitsuka[1], Hayato Kotaki[1], Ryutaro Fukumura[1], Shigeru Makino[1], Yuji Nakai[1], Hideaki Toki[1], Hiromi Motegi[1], Hideki Kaneda[1], Tetsuo Noda[1], Shigeharu Wakana[1], Yoichi Gondo[1]
[1]RIKEN BRC, Tsukuba, Ibaraki, Japan, [2]RIKEN GSC, Yokohama, Kanagawa, Japan

ENU-based mouse gene driven mutagenesis, yet-another reverse genetics, is based on the two key resources; mutant genome DNA and frozen sperm of thousands of ENU-mutagenized G1 male mice. By high-throughput screening of mutant genome DNA library, we can obtain allelic series of mutations on the target gene. Despite of randomly induced mutations, we can usually discover “null” mutation(s) together with many missense mutations. Compared with knockout method, it is efficient to find multiple mutations even in a cost effective way. The targeted mice are recovered by IVF technology from the frozen sperm. This distinctive infrastructure has been widely opened to the community since 2002.
Beta-catenin is unique bifunctional protein. One is a structural protein, and the other is a signaling molecule, which transmits the extra-cellar Wnt signal to the downstream genes via transcriptional activation. We have screened of ~half of entire exons of this gene and obtained twelve point mutations, including one nonsense and six missenses. Then one nonsense (C419Stop) and three missenses (T42I, V195E, and C429S) were recovered. During breeding,, appearance, pathology, blood conditions, and segregation ratio of each genotype were examined. So far several abnormalities were observed, for example, the hyperplasity was seen in the stomache of hetro- and homozygotes of the T42I mutation, also reported in rather few cases of human cancer. Parallel to mouse phenotyping, molecular phenotyping was examined. The expression vectors for all mutant cDNA were constructed. Now we are testing the molecular property of mutatnt proteins, such as transcriptional activity and cellar localization.

P97

IDENTIFICATION OF TESTICULAR GERM CELL TUMOR SUSCEPTIBILITY GENES FROM THE 129.MOLF-CHR19 CONSOMIC STRAIN.

Rui Zhu, Shirley Hammond, Sara Ali, Angabin Matin
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Predisposition to testicular germ cell tumors (TGCTs) is a complex genetic trait. Multiple genetic loci and their interactions contribute to TGCTs in humans and mice. TGCTs originate during embryonic development from transformed primordial germ cells in the gonads of tumor susceptible mice. The 129.MOLF Chr 19 (or M19) consomic strain has high TGCT incidence. As a prelude to TGCT gene identification, we mapped multiple loci on mouse Chr 19 that independently and epistatically contribute to tumorigenesis. To identify TGCT candidate genes, we analyzed gene expression profiles of gonads from M19. Two genes, *D19Bwg1357e* and *Sf1,* whose expression levels were changed in M19, also mapped to two previously delineated TGCT loci. To further evaluate TGCT candidate genes, we have generated mice with targeted deletions of specific genes. In one study, we inactivated *Sf1* using gene trap clones. We found that deficiency of *Sf1* protects against TGCT development. *Sf1* (splicing factor 1) is involved in spliceosome assembly. Our hypothesis is that *Sf1* regulates alternative splicing of pre-mRNA in germ cells and deficiency of *Sf1* may decrease alternative splicing (and production of oncogenic variants) and thus decrease incidence of TGCTs. Much of the genetic and environmental factors that cause TGCTs are poorly understood. Using the approaches described, we demonstrate that the M19 strain has been invaluable for unraveling the complex genetics of TGCT development.

P98
A NOVEL GENE AFFECTING CELL CYCLE PROGRESSION

Michael E. Rusiniak, William C. Burhans, Rosemary W. Elliott
Roswell Park Cancer Institute, Buffalo, NY, United States

We have been searching for genes affecting colon cancer susceptibility and have shown that a 10 Mb region on mouse Chromosome 12 contains at least one such gene. Analysis of candidates in the region uncovered a novel gene that is involved in cell cycle control. Sequencing the susceptible (ICR/Ha) and resistant (C57BL/6Ha) strains for cDNA from this locus showed base changes in both the coding and untranslated regions. No difference in the mRNA level was found using quantitative PCR when comparing colons from the two strains. To determine the function of the gene we used human shRNA clones in a retroviral vector to knock down expression of the human homolog in the human adenocarcinoma cell line HT-29 and isolated a stably infected line. The specific mRNA in the mutant line (KD-1) was reduced to 35% of the HT-29 cells infected with vector alone (EV-1). Cell proliferation measured by BrdU incorporation was reduced to about 60% of the control line (EV-1). These lines were used for cell cycle analysis. There was a decrease in G1 cells from 57% of the total in EV-1 to 20.4% in KD-1. Cells in G2 were increased from 7% to 21% in KD-1, while cells in S phase were increased from 36% to 58.5%. Therefore decreasing the expression level of the candidate gene slows passage through S phase and G2/M. This suggests that the protein encoded by the candidate gene is required for efficient DNA replication. Supported by NIH grant CA115436 to RWE.

P99
DOMINANT MUTATION OF *GSDMA3* IS INVOLVED IN MULTI-STEP SKIN TUMOR DEVELOPMENT

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Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are major forms of non-melanoma skin cancers. It is widely accepted that tumorigenesis, especially SCC, is a multi-step process that involves sequential genetic alterations including mutations of tumor suppressor genes in early event. However, molecular basis of SCC development is still poorly understood. Recently, we reported novel gene family, Gasdermin (*Gsdm*), which are specifically expressed in the epithelial cells in skin and gastrointestinal tract. Based on the expression patterns and chromosomal locations of human *GSDM* family genes, we inferred that function of *GSDM* family genes is associated with tumor development. Gasdermin A3 (*Gsdma3*), one of the mouse counterparts of human *GSDMA*, is responsible gene for a dominant mouse mutation, Recombination-induced mutation 3 (*Rim3*), which exhibits hyper-proliferation and mis-differentiation of the epidermis. To assess involvement of *Gsdma3* mutation in tumorigenesis *in vivo*, we examined skin tumorigenicity of the *Rim3* mutants. *Rim3* rarely develop skin tumors, suggesting that *Gsdma3* mutation alone is insufficient to develop skin tumor. Hence, we introduced the *Rim3* allele onto genetic background with a mutation of tumor suppressor gene to generate a model of multi-step tumorigenesis. We found that the double mutant mice frequently developed multiple SCC with metastatic tumor foci in the cervical lymph nodes. These data suggested that dominant mutation of *Gsdma3* is one of the possible events in the multi-step SCC development.

P100
ESSENTIAL ROLE OF *NANOS3* FOR ACQUISITION OF GERM CELL IDENTITY IN MICE

Satoru Kobayakawa[1], Yumiko Saga[2], Kuniya Abe[1]
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In *Drosophila*, *nanos* is required for abdominal segmentation as well as for germ cell development and maintenance. Conserved functions in germ cell development have been reported for *nanos* orthologs in *C. elegans*, zebrafish, and mouse. *Nanos3* is one of the *nanos* genes found in mice. In Nanos3-null embryos, initial population of primordial germ cells (PGC) are formed, but PGCs are eventually lost during development, leading to sterility in both sexes (Tsuda et al., 2003). This germ cell loss in *Nanos3*-null PGCs is partially explained by apoptotic death of PGCs (Suzuki et al., 2008).
Present study shows that founder population of PGCs at the base of allantois in E7.5 embryos was not fully established in *Nanos3*-null mice. Normally, the PGC founders increase their numbers after E7.5 while migrating toward genital ridges, but the number of the *Nanos3*-null PGCs did not increase significantly from the early bud stage onward. More important, we found dissociation of PGC markers in the *Nanos3*-null PGCs; *stella/PGC7*, *OCT3/4* protein, and *Oct3/4□delta-PE-GFP* (reporting the activity of distal enhancer of *Oct3/4*) are co-expressed in most (>90%) of the wild-type PGCs at around E8.5, whereas only ~30% of the *Stella*-positives were *Oct3/4□delta-PE-GFP* -positive in *Nanos3*-null PGCs. Interestingly, such discrepancy can be found even in *Nanos3*$^{+/-}$ heterozygous PGCs.
Other peculiar feature of migrating PGCs is epigenetic reprogramming, in which histone modification and global DNA methylation are dynamically changed, but *Nanos3*$^{-/-}$ PGCs showed altered epigenotype. For example, although PGCs usually undergo global DNA de-methylation, some of the mutant PGCs maintained relatively high level of DNA methylation. Level of H3K4me3 modification associated with transcriptionally active state is progressively reduced in normal PGC development but not in *Nanos3*-null PGCs.
These data suggest that there exist PGC-like cells expressing some of the PGC markers in the *Nanos3*-null embryos, but their development seems incomplete, which may cause germ cell loss in the mutant. Further study on the aberrant phenotype of *Nanos3*-null PGCs should facilitate dissection of molecular pathways operating in PGC specification and development in mice.

P101
THE MEDIATOR COMPLEX PROTEIN MED31 IS REQUIRED FOR MAMMALIAN DEVELOPMENT

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We have identified a mouse mutant, *L11Jus15*, from a balancer chromosome mutagenesis screen. This mutant exhibits growth defects and late-gestation lethality. Meiotic mapping and positional cloning demonstrates that this phenotype results from a mutation in the Mediator complex gene *Med31,* which has not previously been mutated in mice. The Mediator complex serves as a bridge between gene-specific activators and the RNA polymerase to initiate transcription from Pol II promoters. In addition, individual Mediator complex proteins may interact with distinct transcription factors to activate the transcription of specific target genes. Med31 was first identified in yeast, although it is not essential in *S. cerevisiae*. In *Drosophila, Med31* is required for anterior-posterior axis formation. We have found that in the mouse *Med31* is an essential gene, required for embryonic growth. *L11Jus15* mutant embryos have fewer proliferating cells than controls, especially in regions such as the forelimb buds that expand rapidly during development. Likewise, fibroblast lines created from mutant embryos have a severe proliferation defect, and abnormal levels of cell cycle proteins. The contrast between the phenotype of our mouse mutant and those of *Drosophila* and yeast *Med31* mutants suggests that despite the high evolutionary conservation of Mediator complex proteins, *Med31* has different functions in different organisms. As the Mediator complex is a transcriptional co-activator, the *L11Jus15* mutant phenotype suggests that *Med31* activates the transcription of genes required for embryonic growth and cell proliferation. This is the first time such a role has been proposed for a Mediator complex protein.

P102
DEVELOPMENT OF A NOVEL ASSAY TO MAP ANGIOGENESIS QTLS IN PERINATAL MICE.

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Complex traits pose significant challenges to the conventional methods that are used to positionally clone mutations in mice. While significant phenotypic differences are readily observed in parental strains, segregation of multiple loci with small additive effects in the mapping population can scuttle the most valiant efforts to identify the underlying genes.

The identification quantitative trait loci that control angiogenesis has been limited by the lack of robust assays that can be run on the large populations. While *in vitro* approaches do not represent the complexity of angiogenesis the *in vivo* methods must differentiate neovessel formation from established vasculature.

We have developed a novel quantitative ex-vivo skin biopsy method to quantify angiogenic response in mice to Vascular Endothelial Growth Factor. Our approach combines the complex tissue interactions of the in vivo methods with the speed and reproducibility of the in vitro approach. The advantages include increased throughput, minimal mouse colony expense and the ability to measure small differences in blood vessel growth that are potentially controlled by fewer loci than whole animal studies.

Our preliminary findings contrast previous studies using *in vivo* methods because FVB/NJ mice produced the highest amount of blood vessels when compared to other inbred strains. A [B6XFVB]F1 X FVB backcross was performed to localize the VEGF sensitivity QTLs. We were able to find three QTLs controlling our trait. One of these QTLs localizes to a previously detected angiogenesis QTL. Ongoing experiments are investigating the potential sources of blood vessel progenitor cells and the role of keratinocytes in endothelial cell growth and migration.

P103
GENETIC BASIS OF NEURAL CREST DEFICIT IN DOWN SYNDROME MICE

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Craniofacial abnormalities are distinguishing features found in all individuals with Down syndrome (DS). Trisomy 21 leads to facial skeletal abnormalities including brachycephaly, shortened midface, and small mandible. The Ts65Dn mouse model of DS has three copies of about half of the genes on human chromosome 21 (Hsa21) and exhibits DS-like alterations in craniofacial structure including a small dysmorphic mandible. We have established that the neural crest (NC), a developmental precursor of the craniofacial skeleton, is altered in early development of Ts65Dn embryos. The mandibular precursor, the 1st pharyngeal arch (PA1), is smaller and contains fewer NC at midgestation in Ts65Dn compared to euploid embryos. NC generation, migration, and proliferation are altered in Ts65Dn development.
To understand how trisomy causes the NC deficit, we are examining gene expression in trisomic and euploid NC and alterations in the craniofacial precursors of the Ts1Rhr trisomic mouse model. Ts1Rhr mice are trisomic for a subset of genes found in Ts65Dn but display different craniofacial dysmorphology than is associated with Ts65Dn mice or trisomy 21, including a larger dysmorphic mandible. The PA1 from Ts1Rhr midgestation embryos is larger and contains more NC than euploid or Ts65Dn embryos. Since the Ts65Dn and Ts1Rhr mouse models have ~33 trisomic genes in common but display different effects on skeletal precursors, interactions between these genes and others found on Hsa21 are important in the development of craniofacial abnormalities. Understanding how trisomic gene expression causes NC changes early in development will help determine the genetic basis of craniofacial and other DS phenotypes.

P104
MULTIDIRECTIONAL EFFECTS OF SINGLE – TRAIT, LONG TIME SELECTION OF MICE INBRED LINES.

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Inbred lines L (light line) and C (heavy line) were derived from a highly heterogeneous population was produced through four inbred strains - A/St, BN/a, BALB/c and C57BL/6J intercrossing. Chosen mice were selectively bred for 127 generations, for the low (L) and high (C) body weight at weaning. As a result of long-term selection, the mice from two lines varied significantly in body weight (6g in L line vs. 15g in C line at weaning). The differences regarded also maturation rate, the length of the life time, reproduction performance, fertility and behavior. The very first results of QTL analysis of the reference family derived from the intercross of light (L) and heavy (C) lines revealed existing of QTLs that may contribute to body composition parameters and behavioral phenotype. The small effect of identified QTLs suggests poligenic model of inheritance of the analyzed traits. Additionally, it has been indicated that alterations in gene and genotypes frequencies under selective pressure affect a limited number of microsatellite loci.

P105
***PRDM9*, A POSITIONAL CANDIDATE FOR THE MOUSE HYBRID STERILITY 1 GENE**

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Identification of genes contributing to speciation barriers remains a challenge. The aim of this study was the positional cloning of the Hybrid sterility 1 gene (*Hst1*), on mouse chromosome 17. The gene participates in a breakdown of spermatogenesis in F1 crosses between some laboratory strains (e.g., C57BL/10 or B10) and certain *Mus musculus musculus* mice, such as of the PWD strain. Other hybrid males, e.g. (PWD X C3H), are fertile. The *Hst1* gene has been previously mapped to a 360-kb region on a high-resolution ((B10-*T* x C3H)-*T* x B10) backcross.
We combined genetic mapping, allelic sequencing, haplotype mapping, expression profiling, and transgenic rescue by C3H bacterial artificial chromosomes (BACs) to isolate *Hst1*. The testicular cells of sterile and fertile hybrids were studied by immunofluorescence assays and by real-time RT-PCR. The candidate region for *Hst1* was narrowed down to a single gene, *Prdm9*, and the gene confirmed by comparing the phenotypes of its null allele with the phenotypes of sterile hybrids.
In conclusion, we identified the first candidate for a vertebrate gene contributing to a speciation barrier.

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P109
MRC HARWELL – A CENTRE FOR GENE-DRIVEN MUTATION DETECTION

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Gene-driven screens of mutagenised mice represent a powerful tool in the identification of allelic variants. Male mice, exposed to ENU (N-ethyl-N-nitrosourea) have been used to create a mutant resource consisting of two parallel archives of frozen sperm and DNA taken from over 7000 F1s. Screening of the archive can be used to identify an allelic series for any given gene of interest. Once a mutation is identified through high throughput DNA screening, the mutant mice can be rederived easily by *in vitro* fertilisation (IVF) for phenotyping. As ENU induces point mutations it can be expected that the full range of functional changes in any gene might be uncovered, including amorphs (null), hypomorphs, hypermorphs and neomorphs.

Over 190 Mbp of coding sequence has been screened, generating an approximate hit rate of 1 mutation identified per 1.4 Mbp.

We have performed several screens using DHPLC on the Transgenomic WAVE, TGCE on the SpectruMedix, and more recently Hi-Resolution DNA melt analysis on the Idaho Technology LightScanner. Hi-Res DNA melt analysis is now our preferred method of mutation detection, offering us the potential of performing whole DNA archive screens against several fragments within a few weeks. Our DNA is available to academics as a community resource; alternatively, we are now offering full training to visiting scientists to perform gene-driven screens within our mutation detection facility at the MRC Harwell.

http://www.har.mrc.ac.uk/services/dna_archive/

P110
A NONSENSE MUTATION IN THE MOUSE *ZDHHC13* GENE IS RESPONSIBLE FOR THE *LUCA* PHENOTYPE

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In this study we present *luca* (*luc*), a new mouse mutation associated with hair loss. The affected homozygous mice develop generalized hypotrichosis and multifocal patchy alopecia, starting at postnatal day 7. Hair shaft dysplasia is characterized by twisting and multifocal pigment clumping, suggesting defects in the complex differentiation program of trichocytes. At postnatal day 22, the number of anagen II hair follicles appears to be reduced in homozygous mice, whereas in wild type and heterozygous mice all hair follicles undergo their first true anagen phase. At postnatal day 30, all hair follicles in the mutant mice have very prominent sebaceous glands, suggesting that anagen induction occurs in tylotrich hair follicles only, whereas non-tylotrich hair follicles likely remain in telogen or undergo atrophy. Multifocal alopecia and erythema in homozygous mice are also associated with inflammatory cell infiltration. A basic immunological screen showed no differences between *luc/luc* mice and littermate controls.

We determined that *luc* is an autosomal recessive mutation localized to chromosome 7, between markers D7Mit170 and D7Mit272 (54.6 – 56.1 Mb), a region homologous to human chromosome 11p15.1. During the sequencing of candidate genes we have identified a missense mutation in exon 7 of the *Zdhhc13* (zinc finger, DHHC domain containing 13) gene. This T-A substitution, which results in a premature stop codon, is expected to generate a truncated form of the ZDHHC13 protein. *Zdhhc13* is a member of the palmitoyltransferase family of genes that catalyzes posttranslational modifications in proteins and also may be involved in the NF-kappa-B signaling pathway. Members of this family of DHHC genes have been linked to mental retardation and schizophrenia in humans. Our data shows that the *luca* mutation represents a potential new model for alopecia, hair follicle cycling and trichocyte differentiation. To our knowledge, this is the first report of a mouse model with an external phenotype due to a defect in this member of the DHHC palmitoyltransferase family.

P111
WWW.FINDMICE.ORG -- FIND MICE, KNOCKOUTS, GENE TRAPS, MUTANT ES CELL LINES…

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Challenge: Find the right mouse resources and disease models for my basic and translational research projects.

Answer: Use www.findmice.org, the website for the International Mouse Strain Resource (IMSR).

The IMSR database unifies information about mouse resource holdings worldwide, including inbred, mutant, and genetically engineered mice maintained as breeding stock; cryopreserved embryos, ovaries and sperm; and ES cell lines. Mouse repositories in the U.S., Canada, Europe, Japan, and Australia regularly contribute data on their current stock and ES cell line holdings.

Users can search IMSR for mouse resources based on allele, gene, or strain names, IDs, type of mutation carried, and type of resource (e.g., live, cryopreserved, cell line). In addition, searches can specify repository(s) or regional location. Results include official strain names, links to strain data profiles and mutant descriptions, and contacts for ordering. New repository sites, including those from the International Knockout Projects are being added and new features to improve IMSR searches are being developed.

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P112
MICE GENOME STRESS IN THE CHERNOBYL SIMULATION EXPERIMENT

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South districts of Belarus are still highly radiocontaminated even after 22 years from the Chernobyl catastrophe in 1986, and consequent environmental changes are stored in the soil, plants and animals. The major radionuclides in the contaminated areas are ^{137}Cs and ^{90}Sr, and their physical half-lives are 30.2 and 28.9 years, respectively. It is easily predicted that the radionuclides are concentrated by the food chain into the living organisms in the contaminated area, and radionuclides remain in the irradiated organisms not only externally but also internally for long periods.
We measured the ^{137}Cs radioactivity and its distribution in mice in the middle contaminated area (Babchin). The ^{137}Cs radioactivities of mice in 1997 and 2005 were 14.2-92.7 Bq/g and 1.1-7.6 Bq/g, respectively.
To simulate the radiocontamination in middle contaminated areas of Belarus (1997), mice were maintained for 8 months in the radioisotope facility with free access to drinking water containing ^{137}CsCl (10Bq/ml and 100Bq/ml). Mice were assessed the long term low dose rate and low dose internal and external radiation effect by the quantitative measurement of the contaminated radionuclides-induced DNA double-strand breaks by γ-H2AX foci in the organs.
(The work was supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education, Culture, Science and Technology)

P113
A RECESSIVE ENU SCREEN FOR NEW DEAFNESS LOCI

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G3 mice were screened for hearing loss and vestibular dysfunction, using a calibrated clickbox (a 90dB SPL, 20KHz tone burst) and abnormal behavioural observations, in the Harwell ENU mutagenesis pipeline. G3 pedigrees were generated by backcrossing G2 mice to the G1 founders in each case. A total of 5238 mice have been screened across 158 pedigrees, resulting in the identification of 26 pedigrees with an aberrant clickbox response. Auditory brainstem recording (ABR) has been utilised for physiological confirmation of hearing loss and to determine threshold levels. Structural and ultrastructural analyses of ear structures has been carried out to isolate the cause of the hearing loss in each case. In the pedigrees analysed so far, deafness can be attributed to a variety of causes, including otitis media and ganglion cell loss. In some cases the causative gene has been identified. Interesting examples of new recessive lines and their cochlear pathologies will be presented at this meeting.

P114
GENETIC AMALYSES OF INHERITED RETINAL DEGENERATION MODEL MOUSE IN ENU MUTAGENESIS

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Retinitis Pigmentosa (RP) is one of the genetically heterogeneous disorders characterized by progressive night blindness and loss of peripheral vision resulting from degeneration of photoreceptors. In RIKEN mouse ENU-mutagenesis program, our aim is to establish valuable animal models for RP as a result of identification of the responsible genes and characterization of the detailed phenotypes.
Funduscopic examination was carried out on about 2,500 individuals for dominant screening, and 42 pedigrees (about 800 individuals) for recessive screening. As a result, 36 individuals in dominant screening and 4 pedigrees in recessive screening, showed various abnormal ocular phenotypes.
Three inherited mutants exhibited progressive retinal degeneration which is equivalent to human RP. Among three mutants, the degree of progressive retinal degeneration was different. As a result of positional candidate gene approaches, we identified the point mutation in *Rom1* for the mutant showing slowly progressive retinal degeneration. One of the other two mutants showing fast progressive retinal degeneration was discovered in C3B6F1 G1 founder mice, and had the mutation in *Pde6b* for ENU-injected G0 (B6) origin allele.
So far Rom1 mRNA level and sequences were analyzed in the *Rom1* mutant, but no difference was detected among wild, hetero, and homo genotypes. We report the recent analysis results of the three mutant lines to examine the relationship between the ENU-mutated genes and the retinal degeneration phenotypes in each line.

P115
HIGH THROUGHPUT MOUSE KNOCK-OUT VECTOR DESIGN

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A designing pipeline has been established to generate mouse KO-vectors for the KOMP and EUCOMM projects. Three different types of alleles are produced depending on the structure of the target genes. 1) *Frameshift conditional alleles* target one or few exons in the first half of the coding sequence resulting in a coding frameshift. Ideally the resulting KO-mRNAs are subject to NMD so that no protein can be produced. 2) *Domain-disruption conditional alleles* produce KO-mRNA that are not subject to NMD but a significant part of the protein domain is deleted. Both are based on a targeted trapping approach that generates knock-out first alleles which can be converted to a conditional allele under flip recombinase and then to a null allele under Cre recombinase. 3) Classic *deletion alleles* are needed when a conditional design is not possible.

Candidate designs are generated using a heuristic Perl-pipeline that utilises the EnsEMBL Application Programming Interface (API). The heuristic nature of the pipeline allows for each design to be scored. Candidate designs are presented using a DAS server on the EnsEMBL contig view. Expert annotators choose the best design for each gene and enter them in the vector construction pipeline at an average rate of two 96-well plates/designer/month. First tests suggest that the risk of making designs based on wrong Ensembl gene models is less than 10% and empirical evidence suggests this figure might be strongly reduced by double-checking with the UCSC Genome browser before validating each design.

P116
CHROMOSOME SUBSTITUTION STRAINS AND MOUSE MODELS OF DEPRESSION

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Mouse models of depression are routinely used to assess the sensitivity of novel antidepressants and so mapping Quantitative Trait Loci (QTL) for the phenotype serves to better understand genetic factors underlying the trait. Previously using a BXD panel, several interesting QTLs were identified for immobility in the tail suspension test (TST), which is used as an index of behavioural despair. Few studies have replicated QTLs involved in mapping this trait but given that cross-comparisons have been made with different F2 populations, it is plausible that allelic differences apparent in different inbred strains could be regulating the phenotype. In a collaboration between the Social, Genetic Developmental Psychiatry (SGDP) centre and the Rudolf Magnus Institute (Utrecht), where they have access to the Chromosome Substitution Strains (CSS) C57BL/6J-Chr#A/J/NaJ, a selection were chosen based on QTLs found across the panel of BXD strains, to investigate further QTLs for behaviours in two known models of depression - the TST and Novelty Suppressed Feeding (NSF), another model of depression. Phenotypic data from these tests has revealed that the QTLs found in BXD panel for TST immobility did not capture the major C57BL/6J-A/J difference, and must therefore involve further loci that do not differ between C57BL/6 and DBA. There is however some indication of a C57BL/6J-CSS15 difference across the tests even though each is tapping into alternate endophenotypes of depression. A key question is whether the chromosome 15 QTL governing TST in BXD is synonymous with the CSS15 QTL. Using hippocampal tissue taken from these mice, five biological replicates of each A/J, C57BL6/J and CSS15 have been processed in an exon array experiment to identify if subtle expression QTLs on chromosome 15 can be identified, which accounts for the small C57BL/6J-CSS15 phenotypic effects observed.

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P118
REDUCING ANIMAL NUMBERS: SEQUENTIAL DESIGN OF BEHAVIORAL GENETIC EXPERIMENTS

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Chromosome substitution strains (CSS, also called consomic lines or strains) are strains in which a single, full-length chromosome from one inbred strain – the donor strain – has been transferred onto the genetic background of a second inbred strain – the host strain. We are specifically interested in behavioral genetic research using a commercially available set of mouse chromosome substitution strains (A/J = donor strain, C57BL/6J = host strain). For the consomic strain survey we suggest a limited type of sequential design – the two-stage approach. We propose to start the behavioral tests (modified hole board) with 27 host strain animals and 6 animals per consomic strain. If the P value < 0.05 for the host versus consomic mice comparison, then it makes sense to test extra animals (n = 21) of the appropriate consomic strains. By performing a two-stage approach, a reduction in the number of animals used in the consomic strain survey can be obtained. Our consomic strain survey indicated that nearly all mouse chromosomes each contain at least one QTL that is involved in modified hole board behavior. We have special interest for mouse chromosome 19 because of its more specific association with anxiety-related behavior. Using a fixed sample size design, QTL analysis of male progeny from an F_2-intercross between C57BL/6J and CSS-19 resulted in significant QTLs. It has been reported that a sequential sampling procedure, when compared with a fixed sample size, can provide a substantial decrease in mean sample size required for detecting QTLs. We will elaborate on this.

P119
GENOTYPE × ENVIRONMENTAL INTERACTION IN SOCIAL DOMINANCE DETERMINATION

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Social dominance is important phenomenon in many animal species and its heritable variation has to play a significant role in population processes. To investigate a stability of genetic effects on social dominance, interstrain differences in the social dominance level (a portion of dominant males in the strain) between three inbred mouse strains (BALB/cLac, CBA/Lac and PT) under four various environmental conditions were studied. Paired males of different strains were housing together for 5 days and the social rank of each partner was assessed by asymmetry in agonistic behavior. Used environmental conditions were distinguished to each other by variable degree of familiarity with a territory and also by the presence or not of a female in cages. The results obtained have shown that the environmental conditions were strongly differed in social pressure (a number of fights). As a rule the established social hierarchy was stable for the experimental period. We found significant interstrain differences in the social dominance level, which were not depended on these territory conditions. On the contrary the presence of a female in the cage together with a familiarization of its territory significantly increased a chance to get the dominance rank. However this environmental advantage was not a single crucial factor in social rank determination. Significant genotype × social rank interactions were demonstrated for the agonistic behavioral pattern. We concluded that the genetic effects revealed on social dominance were relatively stable in respective of investigated environmental conditions and could be used for further genetic dissection of social dominance.

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P120
MUFFLED- A NEW MOUSE MODEL OF DEAFNESS

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A deafness screen of mice from the Harwell ENU mutagenesis pipeline has identified a new dominant mouse model of deafness, called Muffled (Mfl). This mutant was identified from the dominant screen as having a severely reduced Preyer reflex in response to a clickbox stimulus (90dB SPL), and a pigmentation defect of a white belly spot and rear feet. The G1 was crossed to C3H females and inheritance was confirmed. The colony was established and continued to be crossed to C3H. Mfl mice have a penetrance of 40% of live mice. ABR testing has identified a variable hearing threshold in the mutants with an average increase in the threshold of 35dB compared to wild type littermate controls. A proportion of the Mfl mice are reduced in size, have tail kinks and exencephaly. Histology revealed a truncated cochlea, malformed ossicles and suppurative otitis media. The cochlea varies in length from a ¼ to a full 1 ¾ turns as studied by Optical Projection Tomography (OPT). An investigation of the hair cells by Scanning Electron Microscopy (SEM) has revealed a variable disorganisation of the outer and inner hair cells. The mechanisms that underlie these defects are under further investigation using various imaging techniques. A SNP genome scan was performed with Mfl mice and this mapped to the distal end of chromosome 11. Candidate genes are currently under investigation.

P121
COMPLEX GENETIC ARCHITECTURE OF SOCIAL INTERACTION AND AGGRESSIVE BEHAVIOR CLARIFIED USING CONSOMIC STRAINS DERIVED FROM MSM AND C57BL/6

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Social behavior has essential role for most animal species. However, genetic mechanisms underlying the individual differences of social behavior have not been well understood because this behaviour is influenced by social interactions between multiple animals. Therefore, genetic analysis of social behavior between same-genotype animals may be more sensitive way as this method will be able to detect both active and passive social behavior as well as interactive enhancement and inhibition caused between same genotype animals. In this respect, consomic strains will be favorable resources to perform genetic mapping of loci related to the social interaction behaviour. We here report genetic mapping of social behavior using inter-subspecific consomic mouse strains, established from MSM/Ms (MSM) and C57BL/6J (B6). Wild-derived strain MSM was highly interactive and showed aggressive behavior in the neutral encounter situation. Analysis of consomic panel of mice revealed chromosomes associated with enhancement (Chr 6, 4, 13 and 17) and inhibition (Chr 8, 9, 11 and 12) of social interaction, and also we identified chromosomes related to aggressive behavior (Chr 4, 13, 15 and 17). Increased aggression in consomic strain of Chr 15 (B6-Chr15^{MSM}) was confirmed in the resident-intruder paradigm. Interestingly, using B6-Chr15^{MSM} as an intruder enhances tail-rattling behavior of B6 resident indicating important role of the intruder genotype for their aggressive behavior. Analysis of a panel of subconsomic strains revealed that tail-rattling and latency for first attack possess different genetic basis even within one chromosome, and both behaviors have contributions from multiple genetic loci that have complex effects.

P122
DEMONSTRATING RESISTANCE-MITIGATING EFFECT OF *ARTEMISIA ANNUA* PHYTOCHEMICAL BLEND WITH *IN-VITRO* CULTURES OF *PLASMODIUM FALCIPARUM* AND *IN-VIVO* WITH *PLASMODIUM BERGHEI* ANKA IN MICE

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Resistance of *Plasmodium falciparum* to drugs such as Chloroquine and Sulfadoxine-pyrimethamine is a major problem in malaria control. Artemisinin derivatives, particularly in combination with other drugs, are thus increasingly used to treat malaria, reducing the probability that parasites resistant to the components will emerge. Although stable resistance to artemisinin has yet to be reported, its emergence would be disastrous due to lack of alternative treatments. The project is to demonstrate resistance-mitigating effects of phytochemical blend of *Artemisia annua* relative to pure artemisinin against *P. falciparum* and on rodent malaria parasite *Plasmodium berghei* ANKA. For the *in vitro* experiments selection will be undertaken on two cultures of *P. falciparum* D6 (CQ-sensitive) and W2 (CQ-resistant), by exposing them to the blend and artemisinin over 50 cycles at doses initially required to give 50% mortality (IC_{50}) of the parasites. Dose-response effects will be determined after 20, 30, 40, and 50 exposure cycles and compared to see if significant difference develops in their efficacy in causing mortality of the parasites. The *in-vivo* experiments mice will be inoculated with the *Plasmodium berghei* ANKA parasite and thereafter given the test drugs. After 4 days the mice will be passaged and parasitaemia determined to calculate the effective doses ED_{50} and the ED_{9O}. The ED_{90} got will be utilized to study resistance under drug pressure in the mice. The nucleotide sequences of the possible genetic modulators of Artemisinin and *A. annua* blend resistance (*mdr1*, *cg10*, *tctp*, and *atp6*) of sensitive and resistant parasites will be compared.

P123
HAVANA MANUAL ANNOTATION AND COMMUNITY INVOLVEMENT

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Manual genome annotation is time consuming, labour intensive and therefore expensive. As such it is only applied to reference genomes such as mouse, human, zebrafish and *C. elegans*. Other genomes, especially those that do not have funding for full-scale annotation, can however still benefit from manual annotation on a selected set of genes via annotation jamborees. The Havana (**H**uman **A**nd **V**ertebrate **A**nalysis a**N**d **A**nnotation) group at the Wellcome Trust Sanger Institute has hosted annotation jamborees for the *Bos taurus* cow and *Sus scrofa* pig genomes. This gave researchers a chance to annotate regions or genes of their interest under expert guidance using the custom written Otterlace annotation system and unified guidelines. Making use of the tools and skills acquired during the jamboree delegates continued to annotate afterwards. On whole genome shotgun sequence such as pig and cow, manual annotation is better at coping with the problems inherent to these types of assemblies: gaps, fragmentation and out-of-order or inverted assemblies. Additionally, detection of pseudogenes and splice variation are key strengths of manual annotation. The Otterlace annotation software is freely available and can be used remotely in conjunction with databases located at the WTSI.

I will present an introduction to our manual annotation system and our experiences using it for the annotation jamborees. I will also discuss selected annotation results such as comparisons with automated annotation.