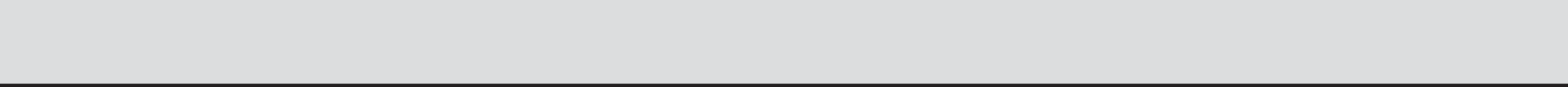


Sunday October 28

Student Satellite Symposium Sessions



Student Satellite Symposium Session 1 Sunday October, 28**1.00 – 1.15pm****S1-1/P186****UNFOLDING POPULATION STRUCTURE AND ANALYSING GENETIC VARIABILITY OF ZALAWADI, GOHILWADI AND SURTI GOAT BREEDS OF GUJARAT(INDIA) USING MICROSATELLITES**Shadma Fatima¹, C. D. Bhong², D.N. Rank³, C.G. Joshi⁴¹MVSc, Dept of Animal Genetics and Breeding, ²Research fellow, ³Assistant professor, ⁴Professor and Head/

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

Key words: Microsatellite, Zalawadi goat, Gohilwadi goat, Surti goat, genetic diversity.**1.15 – 1.30pm****S1-2/P195****GENOME-WIDE ANALYSIS ON ABNORMAL H3K9 ACETYLATION IN ADULT CLONED MICE**Takahiro Suzuki^{1,2}, Shinji Kondo¹, Teruhiko Wakayama³, Yoshihide Hayashizaki^{1,2}¹GSC, RIKEN, ²Int. Grad. School of Arts and Sci., YCU, ³CDB, RIKEN

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

Student Satellite Symposium Session 1 Sunday October, 28**1.30 – 1.45pm****S1-3/P178****IDENTIFICATION OF A NOVEL LOSS-OF-FUNCTION MISSENSE MUTATION IN THE RANKL GENE USING ENU MUTAGENESIS**

Eleni Douni, Eleni Makrinou and George Kollias
Biomedical Sciences Research Center Alexander Fleming

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

We have recently isolated an ENU-induced mouse mutant of osteopetrosis, which is characterized by loss of tooth eruption, abnormally increased bone density, and complete absence of osteoclasts. Genetic analysis using genome-wide polymorphic markers, SSLPs and SNPs, have led to the localization of the causal mutation in distal chromosome 14 at a genomic interval including the RANKL gene. Sequencing analysis of the RANKL coding region revealed a missense mutation, which caused a single aminoacid substitution in a highly conserved region of the extracellular domain.

Functional characterization of the mutated inactive protein provides initial evidence that the mutation affects RANKL binding to its cellular receptor RANK or the decoy receptor OPG. This knowledge provides new possibilities for designing drugs to inhibit RANKL function.

1.45 – 2.00pm**S1-4/P185****PHENOTYPIC EFFECTS OF THE “MINI-MUSCLE” ALLELE IN A LARGE HR x C57BL/6J MOUSE BACKCROSS**

Robert M. Hannon¹, Scott A. Kelly¹, Kevin M. Middleton^{1,2}, Erik M. Kolb¹, Daniel Pomp³ and Theodore Garland, Jr.¹
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From outbred Hsd: ICR mice, we selectively bred four replicate lines for high running (HR lines) on wheels, while maintaining four non-selected lines as controls (C lines). An apparent Mendelian recessive, the “mini-muscle” (MM) allele, whose main phenotypic effect is to reduce hindlimb muscle mass by 50%, was discovered in two HR lines and one C line.

This gene of major effect has gone to fixation in one selected line, remains polymorphic in another, and is now undetectable in the one control line. Homozygotes exhibit various pleiotropic effects, including a doubling of mass-specific muscle aerobic capacity, and larger hearts, livers, and spleens. To begin mapping the genomic location of the MM allele and to better characterize its pleiotropic effects, we crossed females fixed for the MM allele with male C57Bl/6J. F1 males were then backcrossed to the MM parent females. Backcross mice (N = 404) were dissected, and a 50:50 ratio of normal to MM phenotype was observed with no overlap in relative muscle mass. In the backcross, analysis of covariance revealed that MM individuals ran significantly more on days 5 and 6 of a 6-day exposure to running wheels, were smaller in body mass, and had larger ventricles and spleens.

Student Satellite Symposium Session 1 Sunday October, 28**2.00 – 2.15pm****S1-5/P182****QUANTITATIVE TRAITS FOR THE TAIL SUSPENSION TEST: AUTOMATION, OPTIMISATION AND BXD RI MAPPING**

Heena V. Lad, Lin Liu, Josá-Luis Payá-Cano, Cathy Fernandes, Leonard C. Schalkwyk

Social, Genetic and Developmental Psychiatry, Institute of Psychiatry, Kings College London, De Crespigny Park, London SE5 8AF

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

2.15 – 2.30pm**S1-6/P194****IDENTIFICATION OF CONSERVED DNA REGIONS AND A SET OF TRANSCRIPTION FACTORS INVOLVED IN COMBINATORIAL REGULATION OF SEVERAL HUMAN LIVER-ENRICHED TRANSCRIPTION FACTOR GENES**Hisashi Miura^{1,2}, Yasuhiro Tomaru^{1,2}, Misato Nakanishi^{1,2}, Shinji Kondo¹, Masanori Suzuki^{1,2}, Yoshihide Hayashizaki^{1,2}¹Genome Expl Res Grp, GSC/Genome Sci Lab, RIKEN, ²Grad. Sch. of Arts Sci., Yokohama City Univ

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

Student Satellite Symposium Session 1 Sunday October, 28

2.30 – 2.45pm

S1-7/P47

AN ENU-INDUCED MUTATION OF A MIRNA ASSOCIATED WITH PROGRESSIVE HEARING LOSS

Morag Lewis¹, Elizabeth Quint², Agnieszka Rzadzinska¹, Anne Kent-Taylor¹, Helmut Fuchs³, Martin Hrabec³, De Angelis³, Cordelia Langford¹, Stijn Van Dongen¹, Anton Enright¹, Nick Redshaw⁴, Tamas Dalmay⁴, Karen P Steel^{1,2}

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Progressive hearing loss is very common in the human population, but relatively little is known about the molecular or genetic basis. We report here a new ENU-induced mouse mutant called *diminuendo* (*Dmdo*), inherited in a semi-dominant manner. Heterozygotes show progressive loss of auditory responses and hair cell degeneration. Homozygotes show no cochlear responses, have extensive hair cell loss by 4 weeks and show clear head-bobbing. Homozygotes also show anomalies in hair bundle maturation from as early as 5 days after birth, with reduced apical surfaces of hair cells and progressive loss of the staircase organization; however, interstereocilia links appear unaffected at early stages. We mapped the mutation to a 4.5 Mb region of chromosome 6 containing 40 genes, and resequenced the majority of exons within this region.

Eventually, we found a single base change in the seed region of a microRNA, *mmu-mir-96*. This miRNA is expressed in sensory hair cells from an early stage, and we are currently investigating candidates for its target sites to elucidate the mechanism of action.

Student Satellite Symposium Session 2 Sunday October, 28**3.00 – 3.15pm****S2-1/P191****GEMIN2 PLAYS AN IMPORTANT ROLE IN STABILIZING THE SURVIVAL OF MOTOR NEURON COMPLEX**

Chihiro Ogawa^{1,2}, Kengo Usui^{1,3}, Makoto Aoki^{1,3}, Fuyu Ito^{1,3}, Masayoshi Itoh¹, Chikatoshi Kai¹,
Mutsumi Kanamori-Katayama¹, Yoshihide Hayashizaki^{1,2} and Harukazu Suzuki¹

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

3.15 – 3.30pm**S2-2/P193****TRANSCRIPTION REGULATORY CASCADES IN RETINOIC ACID-INDUCED GROWTH ARREST OF HEPG2 CELLS.**

Misato Nakanishi, Yasuhiro Tomaru, Hisashi Miura, Mizue Fujiwara, Masanori Suzuki, Yoshihide Hayashizaki
Division of Genomics, Supramolecular Biology, International Graduate School of Arts and Sciences, Yokohama City University, Laboratory of Genome Exploration Research Group, RIKEN Genomic Sciences Center (GSC), RIKEN Yokohama Institute

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

Student Satellite Symposium Session 2 Sunday October, 28

3.30 – 3.45pm

S2-3/P190

AN N-ETHYL-N-NITROSOUREA MUTAGENESIS SCREEN IN MOUSE IDENTIFIES A CANDIDATE REGION FOR CARDIOMYOPATHY IN THE PROXIMAL END OF CHROMOSOME 1Liliana Fernandez¹, Douglas A. Marchuk², Jennifer Moran³, David R. Beier³ and Howard A. Rockman¹¹Department of Medicine and ²Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham NC. ³Div. Genetics, Brigham & Women's Hospital and Harvard Medical School, Boston MA.

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

3.45 – 4.00pm

S2-4/P183

MAPPING AND CHARACTERIZING ENU-INDUCED MUTANT MOUSE MODELS OF THROMBOCYTOPENIAE. Ricky Chan¹, Heather Lavender², Peter Haviernik³, Kevin D. Bunting⁴, Mark D. Adams⁵Department of Genetics, Case Western Reserve University^(1,2,5), Department of Medicine, Division of Hematology-Oncology, Case Western Reserve University^(3,4), Case Comprehensive Cancer Center, Case Western Reserve University^(4,5)

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

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

Student Satellite Symposium Session 2 Sunday October, 28

4.00 – 4.15pm

S2-5/P192

HAIR-LOSS MUTATION (DEP) CAUSED BY A MUTATION IN PALMITOYL TRANSFERASE ZDHHC21Angela W Lee¹, Pleasantine Mill¹, Masaki Fukata², Ian Smyth³, Margaret Keighren¹ and Ian J Jackson¹¹MRC Human Genetics Unit, Edinburgh, United Kingdom; ²National Institute for Physiological Sciences (NIPS), National Institutes of Natural Sciences (NINS), Okazaki, Japan, and ³Cutaneous Developmental Biology Lab, Monash University, Australia

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

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

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

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

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

4.15 – 4.30pm

S2-6/P187

PSTPIP2 IS MUTATED IN THE FIREWALKER MOUSE TO CAUSE AN AUTOIMMUNE DISEASE

Hwa Jin Baek and Monica J. Justice

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