

MODELING DISEASE AND COMPLEX TRAITS I

ORAL PRESENTATION

Tuesday October, 30

2.30 – 2.45pm

O6,1-1

POSITIONAL CLONING OF THE MOUSE HYBRID STERILITY 1 GENE

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The identification of genes contributing to speciation barriers remains a challenge. Here we describe the positional cloning of the first mammalian candidate for a speciation gene, Hybrid sterility 1 (*Hst1*), on mouse chromosome 17. The gene causes 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. between PWD and 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 with allelic sequencing, haplotype analysis, expression profiling, deletion assay, and transgenic rescue by bacterial artificial chromosomes (BACs) to narrow down the candidate region for *Hst1*. The results of the analyses will be presented.

MODELING DISEASE AND COMPLEX TRAITS I**ORAL PRESENTATION****Tuesday October, 30****2.45 – 3.00pm****O6,1-2****GENETIC ARCHITECTURE AND SYSTEMS PROPERTIES OF COMPLEX TRAITS IN CHROMOSOME SUBSTITUTION STRAINS**

Haifeng Shao^{1,2 *}, Lindsay C. Burrage^{1,2 *}, David S. Sinasac¹, Annie E. Hill¹, Sheila R. Ernest¹, William E. O'Brien³, Hayden-William Courtland⁴, Karl J. Jepsen⁴, Karl W. Broman⁵, Eric S. Lander^{6-8 **}, and Joseph H. Nadeau^{1,2,9 **}

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A meaningful understanding of the genetic architecture of complex traits in most organisms remains elusive because in part we still do not have good estimates of the number of genes that underlie these traits, the magnitude of their effects, or the extent to which they interact. Chromosome substitution strains (CSSs) enable statistically powerful surveys based on testing inbred strains that have single, unique and non-overlapping genetic differences, rather than the customary approach of averaging phenotypic effects across heterogeneous genetic backgrounds. CSSs partition the genome into a panel of inbred strains, each of which has a single chromosome of a donor strain substituted onto the background of a host strain. A survey of 90 blood, bone and metabolic traits in CSS panels of mice and rats revealed a remarkable number of quantitative trait loci (QTLs) that have large and highly non-additive effects. Large phenotypic effects and strong epistasis were also found in congenic panels derived from CSSs. Paradoxically, strong epistasis led to biological systems that were simultaneously robust and fragile. Epistasis usually constrained phenotypic variation and provided robustness to genetic and environmental perturbations, while at the same time single chromosome substitutions tended to shift traits in the parental strains between alternative phenotypic states. Pervasive epistasis has important implications for gene discovery, phenotypic variation, and strategies for treating and preventing dysfunction and disease.

MODELING DISEASE AND COMPLEX TRAITS I**ORAL PRESENTATION****Tuesday October, 30****3.00 – 3.15pm****O6,1-3****ABERRANT NEUROLOGICAL DEVELOPMENT CAUSED BY GENETIC INCOMPATIBILITY BETWEEN TWO WILD-DERIVED MOUSE STRAINS**Juzoh Umemori^{1,2}, Ryouta Kondou^{1,3}, Takeaki Uno⁴, Shigeki Yuasa⁵, Tsuyoshi Koide^{1,3}¹MGRL, Natl. Inst. Genet., Mishima, ²Transd. Res. Integ. Cent., Res. Org. Info. Sys., ³SOKENDAI, Hayama, ⁴Nat. Inst. Info., Tokyo, ⁵Nat Inst Neuro., Tokyo, JAPAN

Many neurological diseases are complex and multifactorial, and the underlying genetic mechanism mostly remains unknown. Furthermore, some of sporadic neurological diseases are believed to be caused by aberrant epistatic interactions of multiple genes. We have been analyzing a mouse model, FURUE mice, which exhibit neurological defects caused by an aberrant genetic interaction, so called genetic incompatibility. FURUE mice indicating pleiotropic defects for example retarded growth, cardiac defect, degenerative eye development, tremor and ataxia. Especially, FURUE showed aberrant myelination, such as reduced number of myelin layers and occasionally incomplete compactons of the most inner layer of myelin sheath in spinal cord and optic nerve. FURUE mice emerged in a F2 population made between two wild-derived mouse strains, BLG2 and KJR. Because these parental strains and the F1 mice never exhibit such neurological and developmental defects, these aberration seemed to be caused by genetic incompatibility of multiple loci between two strains. Through a genetic analysis, we identified one of the causative loci, Genetic incompatibility 1 (Genic1) on Chr13. We have narrowed the Genic1 locus down to definite region between 65.4 to 67.0Mb, less than 1.6Mbp genomic interval, where about forty genes are reported in the database of NCBI build37.1. Interestingly, Genic1 has peculiarly duplicated genomic structure with repeats of gene cluster composed by paralogs of Vmn2r, Zfp, Giot1 and Hmg-1 as well as several unique genes. We are now trying to identify the causative gene/genes in this region to understand the mechanism of the genetic incompatibility.

MODELING DISEASE AND COMPLEX TRAITS I**ORAL PRESENTATION****Tuesday October, 30****3.15 – 3.30pm**

O6,1-4

FUNCTIONAL GENOMICS OF COMPLEX TRAITS WITH INTER-SUBSPECIFIC CONSOMIC STRAINSToshihiko Shiroishi¹, Toyoyuki Takada^{1,2}, Akiteru Maeno¹, Akihiko Mita¹, Kazuo Moriwaki³, Hiromichi Yonekawa⁴¹National Institute of Genetics, Shizuoka, Japan, ²ROIS TRIC, Tokyo, Japan, ³RIKEN BRC, Ibaraki, Japan,⁴Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

An inbred strain MSM/Ms is derived from Japanese wild mice, *Mus musuculs molossinus*. It shows large extent of phenotypic difference in many complex traits, as well as vast amount of genome difference (0.82% SNPs), for a standard laboratory strain C57BL/6J (B6), which is predominantly derived from west European wild mice, *M. m. domesticus*. We have established a full set of inter-subspecific consomic strains, in which each chromosome of B6 is replaced by its counter part of MSM/Ms. Usage of the consomic panel would expand spectrum of target complex traits to be analyzed, because degree of phenotypic difference is larger in the inter-subspecific pair of B6 and MSM/Ms than in any other pair of standard laboratory strains. Beside, inter-subspecific genome difference provides numerous positional DNA markers, which significantly benefits fine mapping of QTLs of interest. We performed systematic phenotyping of the consomic panel, focusing on reproduction-, growth-, and other metabolism-related complex traits. We have successfully detected significant QTLs (at least 169 QTLs in males and 125 QTLs in females), mostly in a sex-dependent manner, acrossing over 40 traits mentioned above. Many of them reflect the differences between B6 and MSM/Ms. It also revealed that quantitative trait measurements often far exceed the range between the above two strains, resulting from allele segregation of the two strains or from disruption of epistasis of multiple genes. All results showed that the established consomic strains would provide a powerful tool to identify latent genetic factors underlying complex traits.

MODELING DISEASE AND COMPLEX TRAITS I**ORAL PRESENTATION****Tuesday October, 30****3.30 – 3.45pm****O6,1-5****USING AN OUTBRED POPULATION OF MICE TO FINE-MAP QUANTITATIVE TRAIT GENES (QTGs)**

Binnaz Yalçın, William Valdar, Richard Mott and Jonathan Flint
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We have fine-mapped an anxiety susceptibility QTL on mouse chromosome 1 to an interval of 0.8 cM using 800 Heterogeneous Stock (HS) mice. We then took advantage of a commercially available strain of outbred mice, MF1, to pinpoint *Rgs2* (Regulator of G protein signaling 2) as a causative gene for anxiety-related phenotypes¹. The validation of this approach prompted us to use an even larger population of HS mice consisting of 2000 individuals and collect additional complex phenotypes in the fields of anxiety, diabetes, obesity and asthma which resulted in the genetic mapping of 843 QTLs². By again using the MF1 population of mice, the number of disease linked genes was significantly narrowed down for our highest QTL peaks. This study provides us with a proof of concept laying the path to improved methodologies allowing the mapping of single genes which candidacy will ultimately be validated by transgenesis work.

1. Yalcin B, Willis-Owen SA, Fullerton J, Meesaq A, Deacon RM, Rawlins JN, Coplev RR, Morris AP, Flint J, Mott R. Genetic dissection of a behavioral quantitative trait locus shows that *Rgs2* modulates anxiety in mice. *Nat Genet.* 2004 Nov;36(11):1145-7.
2. William Valdar, Leah C Solberg, Dominique Gauguier, Stephanie Burnett, Paul Klenerman, William O Cookson, Martin S Taylor, J Nicholas P Rawlins, Richard Mott & Jonathan Flint
Genome-wide genetic association of complex traits in heterogeneous stock mice *Nature Genetics* - 38, 879 - 887 (2006)

MODELING DISEASE AND COMPLEX TRAITS II**ORAL PRESENTATION****Tuesday October, 30****4.30 – 4.45pm**

O6,2-1

HIRSCHSPRUNG'S DISEASE MODEL MICE: MICE WITH DIMINISHED RET EXPRESSION EXHIBIT IMPAIRED NEURONAL SURVIVAL IN THE COLON

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Hirschsprung's disease is one of the most common congenital disorders among humans and is characterized by the absence of the enteric ganglia in the distal gut. Through a recent genetic study we have been able to show that conditional ablation of GFR(α)1, the high affinity receptor for GDNF, in mice during late gestation induces rapid and widespread neuronal death in the colon. This subsequently leads to colon aganglionosis reminiscent of Hirschsprung's disease (HSCR). RET tyrosine kinase functions as the signaling component for the GDNF receptor complex. Mutations in the *Ret* gene potentially affect its transcription level and are primarily involved in HSCR etiology. In this study, we investigated the role of RET in enteric neuronal survival and looked into the involvement of enteric neuronal cell death in HSCR. We generated a novel HSCR mouse model (*Ret*^{9/-} mice) by genetically reducing *Ret* expression levels. These mice displayed colon aganglionosis with incomplete penetrance and sex bias but failed to exhibit any kidney defects, which are all indicative of HSCR. Intestinal aganglionosis develops as a result of both migration delay and compromised neuronal cell survival of enteric neural crest-derived cells in *Ret*^{9/-} mice. Conditional genetic reduction of *Ret* expression levels in postmigratory enteric neurons induced a massive loss in the colon, indicating that diminished *Ret* expression directly affects enteric neuron survival. Our study demonstrates that enteric neuron survival is sensitive to RET dosage and suggests the involvement of neuronal death in HSCR etiology.

MODELING DISEASE AND COMPLEX TRAITS II**ORAL PRESENTATION****Tuesday October, 30****4.45 – 5.00pm****O6,2-2****HESX1 AND TLE3 BLOCK PITUITARY CELL DIFFERENTIATION: LEARNING LESSONS FROM MUTANT MICE**

Luciani Carvalho, Michelle L. Brinkmeier, Mary Anne Potok, Buffy S. Ellsworth, Sally A. Camper
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Pituitary hormone deficiency causes short stature in 1:4000 children born. *Hesx1* is one of many transcription factors that regulate pituitary organogenesis. Humans with mutations in the *HESX1* homeodomain have septo-optic dysplasia and pituitary hormone deficiency (PHD). A patient with a mutation in the engrailed homology (eh1) repressor domain (*HESX1*I26T) presented with evolving PHD including LH, FSH, TSH, cortisol, GH, and PRL. This mutation impairs recruitment of the co-repressor *TLE1* (Carvalho et al., 2003). *PROP1* lesions cause progressive PHD in humans. In mice, *Prop1* represses *Hesx1* and activates *Pit1*, which is necessary for TSH, GH and PRL production. *Prop1* mutant mice exhibit persistent *Hesx1* and ectopic *Tle3* expression, while lesions in *Aes* cause pituitary dysmorphology and growth insufficiency in mice (Brinkmeier et al., 2003). *TLE1*, *TLE3* and *AES* are part of the groucho-related gene family that can bind eh1 to regulate transcription. Due to the overlapping expression of *Tle3* and *Hesx1* in *Prop1* mutants, we tested the functional consequences of *Tle3* and *Hesx1* mis-expression in transgenic mice and cell cultures. These transgenic mice exhibit blocked differentiation of TSH and LH expressing cells, suggesting that *Tle3* and *Hesx1* contribute to the *Prop1* mutant phenotype of failed differentiation. Tissue culture studies reveal that *Tle3* and *Aes* can interact with *Prop1* as co-repressors. Together these studies suggest that molecular analysis of pituitary transcriptional regulation can lead to identification of candidate genes for unexplained cases of growth insufficiency in humans. (NIH R37HD30428)

MODELING DISEASE AND COMPLEX TRAITS II**ORAL PRESENTATION****Tuesday October, 30****5.00 – 5.15pm**

O6,2-3

MODELING ANEUPLOID MOSAICISM IN THE MOUSE USING TARGETED ASSYMETRIC SISTER CHROMATID EXCHANGE (TASCER)

Arnaud Duchon, Patricia Lopes Pereira, Vanessa Besson and Yann Hérault
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Chromosomal mosaicism is a genetic alteration resulting from the missegregation of chromosomes during cellular division. A gain of an additional chromosome in one cell will lead to the trisomy whereas the second daughter cells will be monosomic.

While generating conditional aneuploidies of megabase-long genomic region in the mouse we observed a CRE-mediated asymmetric recombination event leading to monosomic and trisomic cells. This event result from a CRE-induced recombination in between loxP sites locates in a cis configuration that takes place preferentially after DNA replication in various tissues. Such an event was observed using a few regions in the mouse genome and different tissue-specific CRE-expressing transgene. Thus this strategy, called Targeted Assymmetric Sister Chromatid Exchange (TASCER), is a new method available to engineer chromosomes, such as MICER, TAMERE, STRING and simpler approach (for review Brault et al., 2006, Plos Genet., 2, e86; Wu et al., 2007, Nat Genet., 922-930), that opens new perspectives for evaluating the impact of segmental aneuploid mosaicism on the survival of cells in various tissues, and to study the consequences of aneuploidy using mosaic genetics in cancer and other human diseases. In addition, it reinforces the caution that should be taken while trying to generate large deletion in a tissue-dependent manner.

MODELING DISEASE AND COMPLEX TRAITS II

ORAL PRESENTATION

Tuesday October, 30

5.30 – 5.45pm

O6,2-4

THE COLLABORATIVE CROSS: BREEDING UPDATE AT ORNL

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The Collaborative Cross is a cross among eight inbred strains of mice using a combinatorial design to yield many distinct eight-line hybrids that will be inbred to produce recombinant inbred (RI) strains. The goal of this cross at Oak Ridge National Laboratory is to initiate the production of 1680 (8x7x6x5) independent lines, and inbred as many as possible. To maximize the utility of these RI strains, the genetic contributions of each progenitor, and pairwise combinations of progenitors, should be equal when averaged across all hybrid lines. Although each progenitor contributes autosomal genes with equal probability, sex chromosomes and mitochondrial genomes come preferentially from certain progenitors. Because only a subset of all possible eight-way crosses can be performed, contributions of the founders to the resultant population are being brought into balance using a computational approach.

416 funnels were set up in the first phase of breeding. >From these, 391 have reached the G2:F4 (4th generation of inbreeding) and some have reached G2:F10. An additional phase consisting of 400 new funnels has been initiated. Breeding information such as litter size, time to fertility, and infertile lines are also being recorded. To date, loss of lines has been minimal but is expected to increase as inbreeding progresses.

All collaborative cross mice are phenotyped when their grand-progeny are weaned. Following live phenotyping, dissection and construction of a DNA and tissue bank occurs. Trait data and breeding records from the CCDB husbandry database are integrated into the MouseTrack system for heritability analysis and outlier detection.

Supported by The Ellison Medical Foundation and DOE ERKP804.

MODELING DISEASE AND COMPLEX TRAITS II**ORAL PRESENTATION****Tuesday October, 30****5.45 – 6.00pm**

O6,2-5

PROGRESS REPORT AND PHENOTYPIC TRAITS OBSERVED ON THE COLLABORATIVE CROSS MICE FUNDED BY WELLCOME TRUST AND DEVELOPED AT TEL-AVIV UNIVERSITYFuad A. Iraqi¹ and Richard Mott²¹Department of Human Microbiology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel; ²Wellcome Trust Centre for Human Genetics, University of Oxford, UK

This is a progress report describing a project funded by the Wellcome Trust to produce over 100 recombinant inbred mouse lines as part of the Collaborative Cross (CC) genetic reference panel and presents some of the phenotypic traits observed during the inbreeding process. Here, we present phenotypic on coat color, body weight, total Haematocrit, maturity age, litter size and percentage of pre and post-weaned mice of the different lines, from data of more than 100 lines of generations, 1, up to 10 of the cross. Overall, our results show a large variance in body weight for both sexes across the lines. On average, males have tendency to have a bigger body size than females and both sexes exhibit a decrease in weight with generation number. We have observed four major coat colors in the population, white, black, agouti and nonagouti brown, while some diluted colors were also observed. Agouti accounts for over 50% of the population while non-agouti brown is the rarest color. However these mice are highly active and aggressive compared with the others. Litter size and mouse survival at pre and post weaning decrease with the progress of inbreeding, and some lines have low fertility, which agrees with expectation. As of today, the breeding of G8, G9, G10 and G11 generations are in progress with 130 lines in production. We have duplicated the number of lines up to 260 by developing cousins of these lines. An update will be presented at the IMGC meeting.