

MODELING DISEASE BY NATURAL VARIATION/COMPLEX TRAITS**ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****2.00PM - 2.15PM****O37****ALLELIC VARIANTS IN THE CODING AND PROMOTER REGIONS OF TWO TUMOR SUSCEPTIBILITY GENES, P16 AND MTOR, HAVE A COMPOUND EFFECT IN PROMOTING PLASMACYTOMA DEVELOPMENT**

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Susceptibility of BALB/c mice to plasmacytomas is a complex genetic trait controlled by several genes on different chromosomes. We have identified candidate genes for two of the susceptibility/resistance loci on Chromosome 4. *Pctr1*, was mapped to a region that included *Cdkn2a*, which encodes p16INK4a and p19ARF. Mice from which both genes of the *Cdkn2a* locus have been knocked out developed plasma cell tumors over a shorter latency period than the susceptible BALB/cAn strain. Biological assays of p16INK4a and p19ARF alleles from BALB/c and DBA/2 indicated that the BALB/c p16INK4a allele was less active than its DBA/2 counterpart in inducing growth arrest of mouse plasmacytoma cell lines, while the two p19ARF alleles displayed similar potencies in both assays. Promoter variants, which affect p16 expression in BALB/c versus DBA/2 B cells, were identified; a transcription factor, RREB, was found to negatively regulate the p16 gene more effectively in BALB/c mice. Thus, BALB/c p16 has a compound defect that reduces both the level of gene expression and the activity of the protein product. CDK4 inhibitors substitute for functional p16 when tested in vitro. Positional cloning identified *Frap* (mTOR) as a candidate gene for another susceptibility/resistance modifier, *Pctr2*. This locus also encodes an efficiency allele with reduced kinase activity in BALB/c mice, and once again, we have identified promoter variation, which affects its level of gene expression. Knock-in mice carrying the BALB/c allele of mTOR on a resistant background were found to have lower levels of mTOR expression, probably due to neo disruption. Homozygous knock-in mice have several interesting phenotypes that are currently being evaluated. Congenic strains of mice engineered to carry DBA/2 alleles at p16 and Frap were more resistant than mice carrying DBA alleles at only one of the loci. This suggests that the resistance loci act additively to suppress tumor formation. We conclude that plasmacytomas are a model for human cancers, which result from an accumulation of several small defects in efficiency of function, rather than complete loss or gain of function mutations.

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WEDNESDAY NOVEMBER, 15

2.15PM - 2.30PM

O38

FUNCTIONAL GENOMICS BASED UPON MOUSE INTER-SUBSPECIFIC CROSS: WHOLE GENOME SHOTGUN SEQUENCING OF MSM/MS STRAIN AND DEVELOPMENT OF C57BL/6-MSM/MS CONSONIC STRAINS

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MSM/Ms is an inbred strain derived from Japanese wild mice, *Mus musculus molossinus*. Our previous BAC-end sequencing revealed ~0.9% nucleotide difference between MSM/Ms and C57BL/6. Accumulating data indicated that MSM/Ms has unique genetic traits, such as very low tumor susceptibility, wild mice-specific behavioral pattern, thrifty-type energy metabolism and resistance to age-onset deafness. Currently, we are developing an experimental system for elucidation of genome functions to link the SNPs on the MSM/Ms genome and phenotypes characteristic to this strain.

Now, we are conducting whole genome shotgun sequencing of the MSM/Ms genome. As of July 2006, we have finished about 10 million reads of the genome sequence, and have detected ~7.5 million SNPs against C57BL/6. Genome coverage has reached to ~75% of the unique mouse genome sequence. In parallel, we have developed inter-subspecific consomic strains, by substituting each chromosome of C57BL/6 by counterpart of the MSM/Ms chromosomes. At present, 31 strains have been established. For some chromosomes, we have introduced only a part of MSM/Ms chromosome on the C57BL background, splitting one chromosome into two or three pieces, because of high recombination frequency in the cross of C57BL/6 and MSM/Ms. We are collecting phenotype data of these inter-subspecific consomic strains, focusing on the complex traits related to energy metabolism and behavioral pattern. Genome-wide gene expression profiling by microarray analysis is also conducted for a full set of the consomic strains. The results showed marked strain difference in those traits and the gene expression profiling, indicating presence of significant QTL for the relevant traits.

This synergistic experimental system integrating the SNPs data, the inter-subspecific consomic strains and *in silico* MSM/Ms genome BAC cloning system would provide a very unique and powerful approach to study mouse genome functions.

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WEDNESDAY NOVEMBER, 15

2.30PM - 2.45PM

O39

FINE HAPLOTYPE STRUCTURE IN A REGION OF CHROMOSOME 17 IN THE WILD MOUSE

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To elucidate the haplotype structure of the wild mouse, we resequenced 30 loci covering a 252-kb region of proximal mouse chromosome 17. Up to 80 chromosomes were used including ten from *M. m. musculus* and multiple chromosomes from four other (sub)species of *Mus*. Half of the single-nucleotide polymorphisms (SNPs) and even a smaller proportion of insertions/deletions found in laboratory strains were polymorphic also in wild mice. Three *M. m. domesticus*-derived haplotypes were found in twelve Castle's strains, four new haplotypes in other laboratory strains and a more complex structure with short blocks in the wild(-derived) *domesticus* mice. The lengths of the non-recombining blocks deduced from 35 *domesticus* chromosomes of independent origin were tens of kb. The haplotype structure of this region was different in *M. m. musculus*. We suggest that combined phenotyping and genotyping of wild-derived mice may not be useful to identify a candidate region of a trait or a QTL genome-wide, but that it can narrow down a previously characterized candidate region better than thousands of cross animals. The largest non-recombining block encompassed three genes of a highly conserved synteny, thus resembling the haplotype map of the human syntenic region. The haplotype structure of the mouse genome therefore makes the wild mouse a model to study human haplotypes.

**MODELING DISEASE BY NATURAL VARIATION/COMPLEX TRAITS
ORAL PRESENTATION****WEDNESDAY NOVEMBER, 15****2.45PM - 3.00PM****O40****COMPARISON OF THE GENETIC STRUCTURE OF MOUSE POPULATIONS BY COMBINATORIAL ANALYSIS OF LONG-RANGE LINKAGE DISEQUILIBRIUM NETWORKS**R Kirova¹, Y Zhang³, GA Churchill², MA Langston³, EJ Chesler¹¹Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, United States, ²The Jackson Laboratory, Bar Harbor, ME, United States, ³Computer Science Dept., University of Tennessee, Knoxville, TN, United States

Genetic reference populations are defined as panels of related mouse strains with fixed genotypes. Population structure is determined by the breeding history of these populations and particularly, generations of out crossing, randomisation of genotype segregation and progenitor diversity. Using large publicly available SNP sets, we have applied graph analysis to compare the structure of multiple populations and sub-populations. Linkage disequilibrium was evaluated by three metrics: Pearson correlation coefficients, mutual information measures and p-values for Lewontin's D'. A high-pass filter is applied to these metrics to construct an unweighted graph of genotype associations. Maximal complete subgraphs (cliques) were extracted at several thresholds and the resulting graphs were analysed for number of cliques, clique size and chromosomal representation by clique members. These analytic approaches provide a quantitative comparison of populations and can be used to optimise genetic equidistance of sub-populations. Results indicate that the genotype structure of standard inbred strains, which have had longer periods of outcrossing, consists of smaller blocks of linked loci than recombinant inbreds. Moreover, it appears that the non-random breeding history of standard inbreds has resulted in the infiltration of non-syntenic linkage at high LD thresholds. These results are consistently observed across all three LD metrics.

Long-range linkage disequilibrium and the presence of LD blocks in mouse inbred strains have the ability to confound SNP haplotype association analysis, despite the large size of the existing standard inbred strain set. Large syntenic LD blocks in the BXD recombinant inbred strain, though relatively uncorrelated with other genome regions limit the power and precision of this population for genetic analysis. These same limitations apply to other correlation based methods including systems genetic analysis of high-throughput phenotypes such as gene expression. The 8-way collaborative cross is designed to have both smaller LD blocks than existing RI panels, and less long-range (non-syntenic) association of genotypes. Support to EJC and RK: ORNL, managed by UT-Battelle, LLC, under DOE contract #DE-AC05-00OR2275.

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WEDNESDAY NOVEMBER, 15

3.00PM - 3.15PM

O41

GENE EXPRESSION, A PHENOTYPE FOR QTL ANALYSIS

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We have been developing haplotype associated mapping (HAM) algorithms in order to identify QTL for a variety of phenotypes. Currently the SNPster program uses 160,000 SNPs genotyped across 60 inbred mouse strains to infer haplotype patterns across strains and to make associations between haplotype and phenotype.

Expression of all genes in the genome as measured using microarrays can serve as an excellent phenotype in HAM analysis for QTL mapping, and we have measured gene expression variations across a panel of 30 inbred strains for ten different tissues to identify expression QTL (eQTL). Two features are readily apparent from the pattern of significant associations; "cis-acting QTL", which represent instances where the expression level of a given gene is associated with a polymorphism that maps to the gene's location in the genome. These QTL reflect polymorphisms in a gene that influence its own mRNA levels. The second is the presence of strong vertical bands, referred to as "trans-acting QTL". This pattern indicates a single genetic locus that is associated with the expression level of many genes, and presumably one gene (or more) in that locus is a key transcriptional regulator. Trans-acting factors may also include many non-nuclear proteins that influence gene expression through complex molecular cascades, feedback loops and large-scale networks.

Analysis of trans-eQTL bands for overrepresented functional categories using the significance of enrichment of GO terms and/or KEGG pathways enables prioritization of trans-QTL bands in the search for specific regulator genes associated with the expression of downstream targets.