



Friday November 4

1.00pm - 6.00pm

Student Conference Abstracts

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S-1/P-38**SEQUENCE CONSERVATION AT ODZ4, A COMPLEX LOCUS REQUIRED FOR MOUSE DEVELOPMENT, INDICATES THAT MAMMALIAN GENE REGULATION SPANS 450 MILLION YEARS OF EVOLUTION**

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The *Odz4* allelic series is comprised of six different mutants, each with defects in mesoderm development that cause strikingly different phenotypes. The most severe allele fails to undergo gastrulation, while the least severe survives to adulthood with defects in bone formation. This phenotypic pleiotropy foretells the underlying transcriptional, genomic and protein complexity of the 735 kb *Odz4* locus, which encodes more than 20 distinct temporally and spatially restricted transcripts. Using comparative genomics, we sought to identify alternate promoters, 3'UTRs and conserved non-coding sequences (NCS) that regulate the numerous tissue-specific *Odz4* transcripts. We compared 1.3 Mb of genomic DNA flanking *Odz4* from mouse, rat, human, chimpanzee, dog and chicken and identified 428 NCS (>50bp) along this region, with 102 sites conserved to chicken. Strikingly, while 92 NCS identified in mouse/chicken comparisons lie within *Odz4*, only 10 reside in flanking sequences. Twenty-eight of these are >200 bp and show 70% identity between mouse and chicken, emphasizing the high degree of evolutionary conservation found along the *Odz4* locus. Further comparisons with teleosts detected 7 NCS that span 450 million years of evolution. We chose these large, highly conserved NCS for further investigation. Although none contain components suggestive of *cis*-regulatory elements, 3 could harbor alternative 3'UTRs, and we are currently using experimental methods to understand how these sites regulate tissue-specific expression of *Odz4*. Our data reveal that over 100 elements could act coordinately to regulate transcription of *Odz4*, highlighting locus complexity as an evolutionary alternative to one gene, one protein.

S-2/P-39**THE REGULATORY MUTATION, MVWF1, IS A COMMON M. M. DOMESTICUS ALLELE AND THE MAJOR CAUSE OF PROLONGED A PTT IN MICE**

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We previously identified a modifier gene, *Mvwf1*, as the cause of low levels of von Willebrand factor (VWF) in the RIIS/J mouse strain. *Mvwf1* is a regulatory mutation in the gene encoding an N-acetylgalactosaminyltransferase, GALGT2, resulting in a tissue-specific switch in expression from intestinal epithelium to vascular endothelium. Ectopic vascular expression of *Galgt2* results in aberrant glycosylation of VWF, leading to accelerated clearance. Direct sequencing of RIIS/J identified a 30kb region of 2-3% sequence divergence from C57BL/6J flanking exon 1. Analysis of 50 mouse strains identified 10 strains carrying the RIIS/J *Galgt2* allele, all confirmed to exhibit the switch in *Galgt2* expression, including 5 wild-derived strains. Sequencing characterized a common *Mvwf1* haplotype block ~100kb in length encompassing the region of 2-3% divergence. Analysis of DNA from wild-caught *M. m. domesticus* found an *Mvwf1* allele frequency as high as 62% in French mice. These data support an ancient *Mvwf1* founder allele present in a number of mouse strains and retained within the wild mouse population. The aPTT test reflects VWF:FVIII levels and is reported for 43 inbred mouse strains in the Jackson Phenome Database. A survey of the database revealed *Mvwf1* strains account for 5 of the 6 highest aPTTs reported, establishing this single allele as the most common cause of prolonged aPTTs in laboratory mice. The prevalence of this allele suggests that it has been maintained through positive selective pressure, leading us to speculate that a similar survival advantage could account for the high prevalence of VWD in human populations.

S-3/P-97**INTRONIC INSERTION OF A SHORT ENDOGENOUS RETROVIRAL LTR-FRAGMENT IN MGLUR1 GENE DISRUPTS MRNA SPLICING AND CAUSES ATAXIA IN *CRV4* MOUSE MODEL**

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Cervelet-4 (crv4), is a spontaneous mutation which arose in a BALB/c strain at the Institut Pasteur of Paris. Mice homozygous for the mutation exhibit principally a reduced body size and a congenital neurological phenotype, characterized by ataxic gait and intention tremor, with no gross anomalies observed in brain or cerebellum. Linkage analysis allowed us to localize *crv4* locus in the proximal region of chromosome 10, where the metabotropic glutamate receptor type 1 (mGluR1) gene maps. Genetic complementation crosses between *crv4* and mGluR1 null mutant mice confirm that *crv4* is a new spontaneous allele of mGluR1. Molecular analysis of mGluR1 gene in mutant mice revealed the insertion of 190 bp LTR fragment in intron 4. The presence of this LTR fragment caused normal splicing disruption and a complete absence of the wild type protein. On the contrary, a short form of mGluR1 protein, containing only its extracellular part, is predicted and could be hypothetically secreted. Even if null mutants models for mGluR1 gene already exist, *crv4* model, in which the mGluR1 gene carries a splicing mutation with consequent loss of the wild type protein, is of particular interest both because of the mutation mechanism and for the prediction that the truncated protein product might have lost its membrane bound location and acquired a secreted form.

S-4/P-98**DEVELOPMENT AND IMPLEMENTATION OF A VIABLE, PHARMACOLOGICALLY SENSITIZED ENU SCREEN TO IDENTIFY HEMATOPOIETIC MUTANTS IN THE MOUSE**

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The mouse plays an indispensable role in developing our current understanding of mammalian hematopoiesis. Most hematopoietic phenotyping assays are non-viable techniques designed to evaluate homeostatic populations, enumerate progenitor populations, and perform functional analysis. The worldwide effort to generate mouse models of human disease and functionally annotate the mammalian genome using mouse mutagenesis (including dominant ENU screens) requires the development of robust standardized viable phenotyping tools. We have developed a phenotyping assay that induces transient cytopenias using various pharmacological agents (5-fluorouracil, phenylhydrazine, and hydroxyurea), the responses to which are monitored by tracing changes in peripheral blood levels of red blood cells, white blood cells and platelets. We have compared the recovery data with conventional progenitor assays and analyzed a cohort of well-studied hematopoietic mutants using the transient anemia assays that has yielded novel phenotypes of hemizygous mutant animals. Furthermore, we have complemented our dominant generation 1 (G1) ENU hematopoietic screen by implementing a 5-fluorouracil (5FU)-induced cytopenia recovery screen of G1 mice. The 5FU-induced cytopenia recovery assay is also being used as a secondary phenotyping assay for some of our G1 dominant mutants. I will discuss the development and results of our transient cytopenia assay, its use in screening targeted and ENU mutant mice, and several ENU mutants identified in our hematopoietic screens, including a novel mutation in the protein tyrosine kinase Jak2, which leads to thrombocythemia. This point mutation in the protein kinase domain will help us to dissect the recently discovered role of Jak2 in Myeloproliferative Diseases including Essential Thrombocythemia.

S-5/P-99**REGION SPECIFIC ENU MUTAGENESIS ON MOUSE CHROMOSOME 5**

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A region-specific ENU mutagenesis program has been designed to explore the functional content of proximal chromosome (chr) 5 in mouse. A dominant spotting, recessive lethal Rump White inversion (*Rw*) mutation, spanning 30 cM of the proximal region of mouse chr 5, was utilized as a balancer in a three generation breeding scheme. This 52 Mb region, syntenic with portions of human chromosomes 2, 4 and 7, represents about 3% of the mouse genome.

G3 mice, homozygous for the ENU treated chromosome, were screened for various phenotypes. A total of 1103 pedigrees have been screened revealing 43 mutations, 37 of which are embryonic lethal, two are sterility mutations, two are behavioural mutations, one is deafness mutant, and one reduces fitness. Lethal pedigrees were identified by failure to derive test class animals within a pedigree. Embryonic lethal mutations were the largest class of mutations recovered and the time of embryonic death and the corresponding mutant phenotypes have been characterized.

A series of overlapping deletion complexes have been generated across the *Rw* region to facilitate systematic functional analysis and positional cloning of the mutations. By complementation, ENU-induced mutations can be localized to the region of a deletion with a single mating pair. Recombinational mapping was also performed to further narrow the region.

The results of this project present the possibility of region-directed mutagenesis for systematic functional analysis of the mammalian genome.

S-6/P-100**A GERMLINE TRUNCATING MUTATION IN THE RAT ADENOMATOUS POLYPOSIS COLI GENE WITHIN THE HUMAN MUTATION HOTSPOT REGION**

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Much of modern cancer research is predicated on the establishment of tractable and quantitative animal tumor models. Our APC^{Min} mouse is one such model, with a two month growth period for macroscopically visible, countable tumors. Here we report the isolation of a germline truncating mutation of the APC gene in an inbred Fisher-344 line of rats. Genomic DNA from the progeny of N-ethyl-N-nitrosourea (ENU) treated males was screened for truncating mutations in APC using a yeast gap-repair, ADE2-reporter truncation assay. 2530 bases of APC exon 15 were amplified with chimeric primers for APC sequence carrying homology to a "universal vector" that accepts any such chimeric amplicon. The amplicon was then gap-repaired into the universal vector and transformed into ADE2-deficient yeast. Screening of 1360 F1 progeny yielded a single plate with half red, half white colonies; this is the expected ratio for a heterozygous mutant. The mutation was confirmed by sequencing and creates a stop codon at position 1137. It has been transmitted to 40 of 76 offspring to date. This is only the third reported targeted mutant rat and is the first to be generated on a completely inbred line. The addition of the rat model expands the human-mouse tandem to a mammalian triad for colorectal cancer research. The relevance to human disease is further aided by the position of the APC truncation, which recapitulates the mutation hotspot region in human colorectal tumors. The APC^{Am1137} rat will enhance our ability to study the development, progression, physiology and treatment of disease.

S-7/P-101**PHENOTYPIC AND MOLECULAR CHARACTERISATION OF THE DEPILATED (DEP) HAIRLOSS MUTATION**

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Depilated (dep) is an autosomal recessive mutation that is characterised by the loss of hair shortly after birth. It arose spontaneously on a brachyury (T) background at the Jackson Laboratories in 1966. Mutant mice can be identified by an unusually thin and short hair coat as early as a week of age when hairs first emerge from follicles. Previous studies using recombination assays have shown that dep is a deficiency of the epidermis rather than the dermis. We have collected skin and hair samples at 8 weekly time points for more detailed analysis on anatomic changes.

Over the past 50 years, a panel of 25 chromosomal deletions within the 22Mb *brown* (*b*, *Tyrp1*) interval has been generated at the Oak Ridge Laboratories as part of the specific loci test (SLT). In combination with the sequence data generated from BAC contig, the *brown* deletion endpoints have been utilised to define the dep interval to a 160kb region towards the distal end of the *brown* deletion complex which contains 3 genes: *Zdhhc21*, *Frem1* and *Cer1*.

Frem1 is mutated in the classical mutation head blebs and 2 ENU-induced alleles. Complementation analysis between *Frem1* mutants and dep gives normal mice, suggesting that *Frem1* involvement in dep is unlikely. *Cer1* has also been previously knocked out but the null mutants do not exhibit dep phenotype. To date, *Zdhhc21* remains the most probable candidate for dep.

Zdhhc21 is a transmembrane zinc finger protein with DHHC domain from a 21-member family. Sequencing of all *Zdhhc21* exons in dep reveals a single amino acid deletion at residue 233. The possible effects of del233F on protein structure and its interaction with other proteins are being studied using ExPASy tools. To generate an allelic series of *Zdhhc21*, a gene-based screen of two *Zdhhc21* exons are being carried out on an archive of over 5200 DNA samples from individual F1 ENU-mutagenised mice generated at the MRC at Harwell. Each of these samples is paralleled with a frozen sperm sample that can be used to generate mutant backcross progeny for functional analysis and for comparison with dep and *Zdhhc21* null phenotypes.

S-8/P-102**CHARACTERISATION OF SHORTY, AN ENU DERIVED MUTANT MOUSE WITH DEFECTS IN RIB FORMATION**

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We identified a mutant mouse in an ENU mutagenesis screen for late embryonic developmental phenotypes that we call shorty (srt), which has a severe malformation of the vertebrae and ribs. The development of the caudal vertebrae appears grossly normal as do the long bones, suggesting this a patterning problem affecting the thoracic skeleton. The defect is lethal and the affected pups are usually stillborn and smaller in size than wild type littermates. The phenotype of srt is similar to a group of human disorders called spondylocostal dysostosis, characterised by abnormal vertebral segmentation. The phenotype is also similar to that of pudgy (pu), which is caused by a mutation in *Dll3*, a Notch ligand. Our mapping analysis has excluded the possibility that srt is an allele of pu, since the mutation have been localised to a 1.06 Mb region on mouse chromosome 17. The region is within the MHC region and is gene rich. We are currently sequencing 12 candidate genes and preparing an Affymetrix microarray chip comparing srt and wild type RNA. Expression analysis of various genes involved in somitogenesis demonstrates that Pax9 and are less abundant in the mutant compared to wild type. In addition, there appears to be an early developmental delay in the formation of the somites in srt. This novel mutant results in abnormal rib and vertebrae development and could provide insight into the patterning of the ventrolateral sclerotome and somitogenesis.

S-9/P-118**ANALYSIS OF THE NEURAL CREST MUTANT, CHASTITY, IDENTIFIED IN A SOX10 SENSITIZED MUTAGENESIS SCREEN**

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The molecular function of many genes regulating mammalian development has not been elucidated. We have been using a ENU based genetic screen to identify loci involved in the development of the neural crest. This screen was designed to identify dominant mutations that exacerbate neural crest defects observed in mice that are haploinsufficient for the neural crest transcription factor, *Sox10*. *Sox10*^{LacZ/+} mice exhibit small ventral bellyspots due to reduced neural crest derived melanocytes in the skin and are susceptible to megacolon due to reduced neural crest derived enteric ganglia in the gut. One heritable mutant line identified, *Chastity* (*Cty*), displays a significant increase in white spotting compared to the *Sox10*^{LacZ/+} heterozygous mice. *Sox10*^{LacZ/+;Cty/+} mice exhibited extensive ventral spotting that can extend over the dorsal surface to form a belt. Genomic mapping of the mutation localized the mutant allele to chromosome 13 between markers D13Mit158 and D13Mit153. We will present results obtained from the positional cloning of this allele, analysis of the genetic interaction of *Cty* with *Sox10* and characterization of the effects of *Cty* on neural crest development.

S-10/P-119**A DELETION IN THE GENE ENCODING SPHINGOMYELIN PHOSPHODIESTERASE 3 (*SMPD3*) RESULTS IN OSTEOGENESIS AND DENTINOGENESIS IMPERFECTA IN THE MOUSE**

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In man, *Osteogenesis imperfecta* (OI) is an heterogeneous group of inherited skeletal disorders resulting in general from mutations in collagen type 1 genes (*COL1A1* and *COL1A2*). The mouse recessive mutation *fragilitas ossium* (*fro*) is the only mouse model for human severe forms of OI unlinked to collagen defects.

Fragilitas ossium (*fro/fro*), previously known as *forelimb deformity* (*fld/fld*), is an autosomal recessive mutation that was discovered in 1975 in a randombred stock of mice after treatment of spermatocytes with the chemical mutagen tris(1-aziridinyl) phosphine-sulphide (ThioTEPA). This mutation, which often leads to perinatal death, is inherited as a fully penetrant recessive trait. Surviving homozygous mutant mice (*fro/fro*) display most of the features specific to OI : curved and brittle bones, small size and abnormal dentinogenesis, but have otherwise normal lifespan and fertility. The bowing of bones becomes less obvious with age.

Genetic mapping of the *fro* locus was achieved by genotyping 1450+ F2 mice born from two intersubspecific intercrosses involving the MBT/Pas, and MAI/Pas (*Mus musculus musculus*). We mapped the *fro* locus to a 980-kb critical interval on chromosome 8 containing 26 genes. Comparison of transcription profiles of mutant and wild type newborns led us to focus on *Smpd3*, the gene encoding nSMase2 a neutral sphingomyelinase. We identified a large deletion at the 3' end of the *Smpd3* coding frame that led to aberrant C-terminal protein sequence and to complete loss of enzymatic activity.

S-11/P-120**DISRUPTION OF *RAIDD* GENE LEADS TO EARLY PRE-IMPLANTATION LETHALITY OF HOMOZYGOUS EMBRYOS**

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Raidd (RIP- associated ICH1-/CED-3-homologous protein with a death domain) mediates recruitment of Caspase-2 to tumour necrosis factor receptor-1 (TNFR-1) signalling complex. It has been implicated in different pathways since overexpression of *Raidd* induced apoptosis in MCF-7 cells upon TNF α treatment, whereas its overexpression in adipoblasts blocked differentiation to mature adipocytes. Mechanisms by which *Raidd* acts in apoptosis/differentiation are not fully understood yet and we examined this by disrupting *Raidd*'s function replacing exons 2 and 3 with b-gal-*neo* (*Geo*) cassette. Heterozygous mice were normal suggesting that the hemizygous state of *Raidd* is sufficient for normal tissue/organ homeostasis. Staining of heterozygote midgestation embryos for *Raidd*-driven b-gal reporter revealed stage and cell-type specific expression pattern implicating *Raidd*'s role in embryogenesis. However, no homozygous *Raidd/Geo*^{-/-} F₂ adult mice as well as no embryos from E10.5 down to E2.5 pc of pre-implantation development were identified. The ratio of wild type to *Raidd/Geo*^{+/-} remained 1:2, implying that *Raidd/Geo*⁻ mutant allele, when homozygous, causes preimplantation lethality prior to morula stage. However, our attempts to target the remaining wild-type allele in heterozygous *Raidd/Geo*^{+/-} ES cells succeeded, suggesting that loss of *Raidd* is not lethal at the ES cell level (E3.5), but earlier during embryonic development. Hence characterization of *Raidd/Geo*^{-/-} ES cells was performed employing the TNF α induced response. Recently, we were able to generate mice with disrupted *Raidd* alleles with no *neo* cassette and this unexpected result is currently under examination. Overall, our results provide *in vivo* evidence that *Raidd* plays a role during embryogenesis.

S-12/P-121**NEW TRANSCRIPTION FACTORS FOR EARLY EYE FORMATION IN MOUSE**

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Though the developing eye is believed to be a highly specialized extension from the developing neural tube, the formation of major eye structures should be induced via a separate series of coordinated (1) inductive interactions and regional specifications (2) maturation to a functional eye (3) formation of neural connections between retina and optic tectum. Microarrays were used to demonstrate the molecular profiling in the mouse embryonic eye from the optic vesicle formation at E9.5 to the completion of basic eye structure at P0. Differentially expressed transcription factors and signaling molecules in the early developing eye were displayed. Temporal expression patterns were confirmed by quantitative real time PCR and spatial expressions were confirmed by whole-mount *in situ* hybridization. siRNA was used to study their roles in the early eye morphogenesis. The loss-of-function phenotype of these candidate genes was demonstrated by the absence of eye tissues with normal neural tube in the developing mouse embryos *in vitro*. This study revealed that the new neural tube-independent transcription factors and signaling molecules were highly regulated in the induction and formation of optic vesicles in the early eye formation.

S-13/P-122**ZIC2-ASSOCIATED HOLOPROSENCEPHALY IS DUE TO A DEFECT IN PRECHORDAL PLATE DEVELOPMENT**

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Holoprosencephaly (HPE) is the most common defect of forebrain development. Clinical features vary widely; in the more extreme cases these may include cyclopia with a proboscis. Nine genes are linked to HPE in humans and five are members of the *Sonic hedgehog* (*SHH*) pathway. In addition, mutations in *ZIC2* can also give rise to HPE in humans. The *Zic* genes are members of the Gli superfamily of transcription factors and the Gli genes are transcriptional mediators of hedgehog signals. On the basis of homology to, and interactions with, the Gli genes it has been proposed that *Zic2* may act downstream of *Shh* during dorsal-ventral development of the mammalian forebrain. We have isolated a point mutation in the 4th zinc finger domain of mouse *Zic2* which renders the protein unable to bind DNA and ablates the trans-activation ability of *Zic2* in cell based assays. Embryos homozygous for this mutation develop HPE and die at 13.5 dpc allowing the first *in-vivo* study of the mechanism behind *Zic2*-associated HPE. The assumption that *Zic2* functions downstream of *Shh*, predicts that the forebrain expression of *Shh* should be intact in mutant embryos. Unexpectedly, we find that in mutant embryos *Shh* expression in the 9.5 dpc forebrain is down-regulated or absent. At the allantoic bud stages of development, fewer cells express anterior notochord markers and at the streak elongation stage there is a depletion of prospective prechordal plate cells. We conclude that that *Zic2*-associated HPE is due to a defect in prechordal plate development.

S-14/P-123**SEARCH FOR HSF1 OOCYTE-SPECIFIC TARGET GENES**

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Heat shock factor 1 is best known as the transcriptional regulator of the Heat Shock response, which leads to overexpression of Hsp genes in stressed cells. Nevertheless, in absence of any defined stress, HSF1 loss of function in oocyte precludes embryonic development after fertilization indicating that Hsf1 mutation has an important maternal effect and suggesting that HSF1 is necessary to regulate expression of important maternal factors in the oocyte. In order to identify these maternal factors, we decided to exploit *in silico* databases, which are specific for HSF1 and oocyte, respectively. As a first step in this analysis, we have selected known sequences, which appeared in both databases in order to build a subset of genes that are potentially controlled by HSF1 and expressed at high level in oocyte. Consistent with HSF1 regulation, the best candidates should exhibit several putative HSF1 DNA binding sites called HSEs potentially associated with other oocyte-specific regulatory motifs. After having updated HSE consensus sequence, we searched the previously selected subset of genes. So far we have found 41 genes with at least 2 HSEs within 5Kb upstream the transcription start. To further validate these candidate genes, we are taking advantage of interspecies conservation of HSEs and we are comparing their level of expression by RT-real time PCR in Hsf1^{+/+} and Hsf1^{-/-} oocytes. The most differentially expressed candidate genes will be studied for their contribution to the phenotype of embryos derived from Hsf1^{-/-} mothers.

S-15/P-168**UTILIZATION OF INBRED MICE TO IDENTIFY THE FIRST *DISTORTER* OF MEIOTIC DRIVE IN METAZOANS**

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Mendel's law of segregation assumes equal inheritance of alleles at each locus. Transmission ratio distortion (TRD) is a violation of Mendel's law of segregation. We have recently shown directly that TRD at the *Om* (*Ovum mutant*) locus is due to non-random segregation of female chromosomes at the second meiotic division (aka meiotic drive). Meiotic drive is an example of competition between alleles for transmission to the next generation. Every meiotic drive system requires both a *Responder* and a *Distorter* locus. The *Responder* has been mapped to the *Om* locus on mouse *chromosome 11* and the *Distorter* is linked to *Om*. The goal of this project is to identify the first meiotic drive *Distorter* in metazoans. Mapping the *Distorter* has been hindered by the simultaneous presence of TRD and lethality. The later is due to mutation at *Om* resulting in the DDK syndrome. Recently, we have fine mapped the *Om* locus using a panel of experimental males. Using the same panel of males we have now mapped the *Distorter* to different locus and generated males that do not have lethality but do have TRD. This demonstrates that TRD and lethality are separate phenotypes. This work represents the identification of the first *Distorter* of meiotic drive in a metazoan. Importantly, mapping the *Distorter* also demonstrates that the sperm can affect chromosome segregation during female meiosis following fertilization. Lastly, the mapping of this meiotic drive *Distorter* is of interest because its identification will likely bring insight into mechanisms of inheritance and chromosomal biology.

S-16/P-165**GENETIC DISSECTION OF THE MOUSE RESPONSE TO SALMONELLA TYPHIMURIUM USING RECOMBINANT CONGENIC STRAINS**

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Salmonella spp are Gram-negative bacteria that still cause significant disease burden worldwide. Typhoid fever caused by human specific Salmonella serotypes remains an important problem in the developing world while non host-specific serotypes are associated with cases of food-borne gastroenteritis in industrialized countries. The outcome of an encounter between the host and Salmonella spp is determined by several factors including the pathogen virulence, inoculation dose, host immune system state and host genetic background.

In order to understand the contribution of host genes to the outcome of Salmonella infection, a mouse model of Typhoid fever was developed and studied for many years. Using this model, genes with large effect (Mendelian effect) have been identified through positional cloning approaches or gene knockout experiments. However, it is likely that more genes with smaller effects contribute to the host response to Salmonella. In an attempt to identify such genes we undertook a systematic screening of all available AJ and B6 recombinant congenic strains for Salmonella susceptibility. Our hypothesis was that additional genes beside Nramp1 (which is known to be very important in controlling Salmonella Typhimurium infection and also known to segregate in these lines) would segregate in these RCS. This approach allowed us to identify a few strains that showed a deviant phenotype from their known Nramp1 genotype. Two of these strains were chosen to generate fully informative F2 populations. Linkage analysis led to the identification of at least one novel major gene and additional QTLs that influence the mouse response to Salmonella.

S-17/P-177**PYRUVATE KINASE DEFICIENCY AND AN ADDITIONAL LOCUS ON CHROMOSOME 10 CONFER RESISTANCE TO MALARIA IN ACB55 RECOMBINANT CONGENIC MICE**

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We have used a mouse model of malaria with *P.chabaudi* AS to study the genetic factors controlling differential susceptibility to infection in recombinant congenic strains (RCS). AcB55 and AcB61 RCS display unique resistance to blood-stage malaria with low peak parasitemia and 100% survival, despite a largely susceptible background. Phenotypic examination of AcB55/61 revealed extramedullary erythropoiesis in the liver, reticulocytosis and splenomegaly due to increased erythropoietic activity in the spleen. Malaria resistance in these two strains is conferred by a loss of function mutation (*Char4*) in erythrocyte pyruvate kinase (*Pklr*, *Pklr^{90N}*) which results in chronic hemolytic anemia. In addition to *pklr*, linkage studies in an informative [AcB55xA/J] F2 cross detected another locus (LOD = 2.85 at D10Mit189) affecting blood stage replication of *P. chabaudi* AS. This second locus, *Char9*, segregates in a co-dominant fashion, with B6 alleles showing a protective effect on the level of peak parasitemia. Re-analysis of the linkage data in this [AcB55xA/J] F2 cross while controlling for the large effect of *Char4* alleles (*Pklr^{90N}*), results in a significant increase of the LOD score for *Char9* on Chr. 10 (LOD = 4.74, $p < 0.0001$). The transcript map of the *Char9* region contains ~80 genes, including a number of good positional candidates, such as the interferon-gamma receptor (*Ifngr*). Genes mapping to the interval were systematically analyzed for sequence integrity and expression in target tissues, followed by quantitative RT-PCR in AcB55 and A/J spleen, prior to and post infection. We noted a highly significant difference in the expression of one particular candidate, between A/J and AcB55, with little or no transcript detected in A/J spleen or liver. This candidate is being further examined for a possible role in the pathogenesis of or protection against malaria.

S-18/P-178**AGING ALTERS THE GENETIC BASIS OF RESISTANCE TO *BABESIA MICROTI*, A PATHOGEN OF RED BLOOD CELLS**

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Human babesiosis is an emerging infectious disease in New England. Age is the major risk factor for babesiosis, i.e., severe disease is most often seen in individuals over the age of 50. To determine the genetic basis of resistance, we developed a mouse model of infection with a human isolate of *B. microti*. Mice were infected i.p. with 10⁵ parasitized red blood cells. Parasitemia was measured at three-to-four day intervals, and defined as the frequency of nucleic acid positive red blood cells. Two-month old DBA/2 mice developed an intense parasitemia whereas B10.D2 mice were resistant. Male mice from reciprocal (DBA/2 x B10.D2) F₁ crosses failed to develop parasitemia, indicating that resistance is a dominant trait conferred by autosomal genes. Segregation analyses of 141 F₂ mice infected at young age mapped a major locus of resistance (*Babesiosis resistance locus-1*, *Brl-1*; LOD 13.2) on proximal chr.9 that accounted for 38% of phenotypic variance. A weaker linkage was detected on distal chr.4 (*Brl-2*; LOD 4.0). When infected at 18 months of age, DBA/2 displayed a greater susceptibility whereas B10.D2 remained resistant. Segregation analyses of F₂ mice infected at 18 months indicated a lesser role for *Brl-1* and *Brl-2* (LOD <3.0), and revealed a novel locus *Brl-3* (LOD 4.5) on distal chr.9. These studies establish that i) B10.D2 alleles at *Brl-1* and *Brl-2* are determinants of resistance to *B. microti* at young age and ii) aging alters the genetic basis of resistance by recruiting *Brl-3*.

(Supported by National Institute on Aging, NIH, R01AG19781)