AMDCC P&F Progress Report
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Under independent funding, we have carried out an F2 cross between C57BL/6 (B6) males carrying the heterozygous db mutation and DBA/2 females. F1 progeny were genotyped and those carrying the db mutation were selected for breeding to produce the F2 generation. F2 progeny homozygous for the db mutation were selected for phenotyping and tissue collection.
For analysis, we selected two cohorts of F2 animals:
a) 250 animals of both genders at 4 to 5 weeks of age. At this age, we expect strong differences in insulin sensitivity as measured by plasma insulin levels, with most animals showing good glycemic control.
b) 250 animals of both genders at about 12 weeks of age. For these animals, we expected a range of phenotypes from insulin resistant to fully diabetic with a corresponding level of function in the pancreas.

In addition, for each age group, we have collected tissue and phenotype data for 6 B6 db/db and 6 BLKS db/db mice. For this basic experiment, we have extensive phenotype data including measures of body weight and fat mass using nuclear magnetic resonance as well as plasma levels of glucose, insulin and many other relevant analytes.. For gene mapping, we will analyze the DNA for approximately 2000 informative SNP's using the Affymetrix 5K mouse chip. We have collected a variety of tissues for expression analysis including pancreatic islets, muscle, liver and adipose tissue. These tissues will be used for integrated genetic and network analysis to characterize genes related to the core phenotype of diabetes susceptibility.

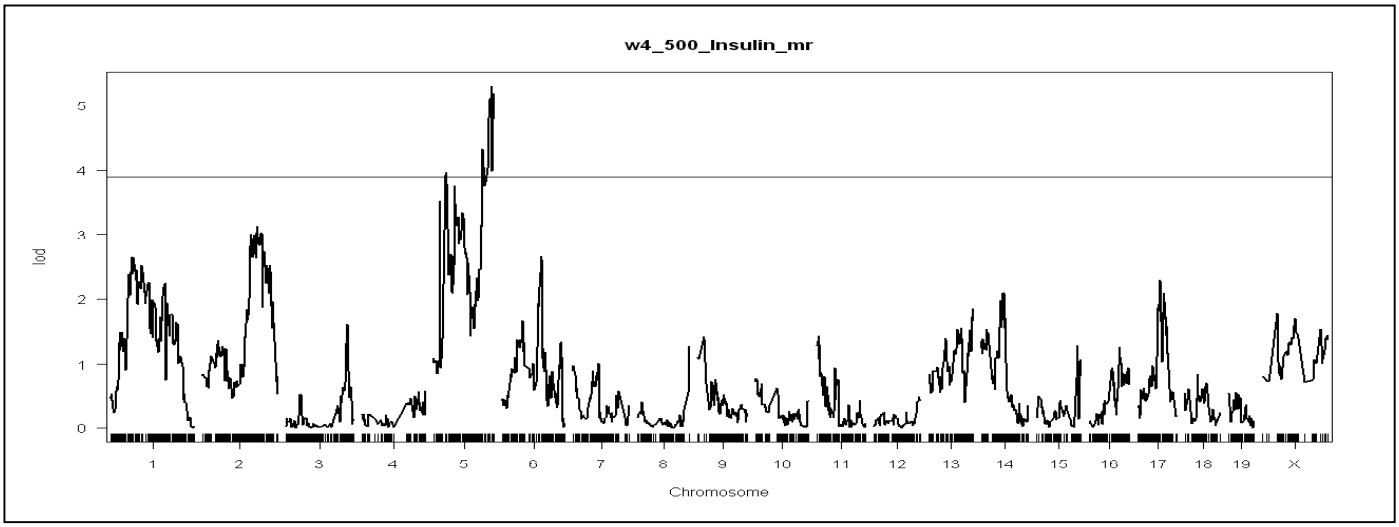
For the present proposal, we have collected hearts and kidneys from all animals in the cross. In the context of the Pilot and Feasibility grant, we proposed to profile general RNA expression in heart and kidney from 100 of the 12 week animals as one measure of obesity-induced diabetes and its complications. As physiologic measures of diabetic complications in heart and kidney, we proposed to carry out histological assessment in the second kidney and in half the heart. In addition, we collected plasma and urine samples from each animal for use in determination of kidney function as reflected in urine albumin/creatinine ratios determined by ExoCell kits and in blood urea nitrogen levels.

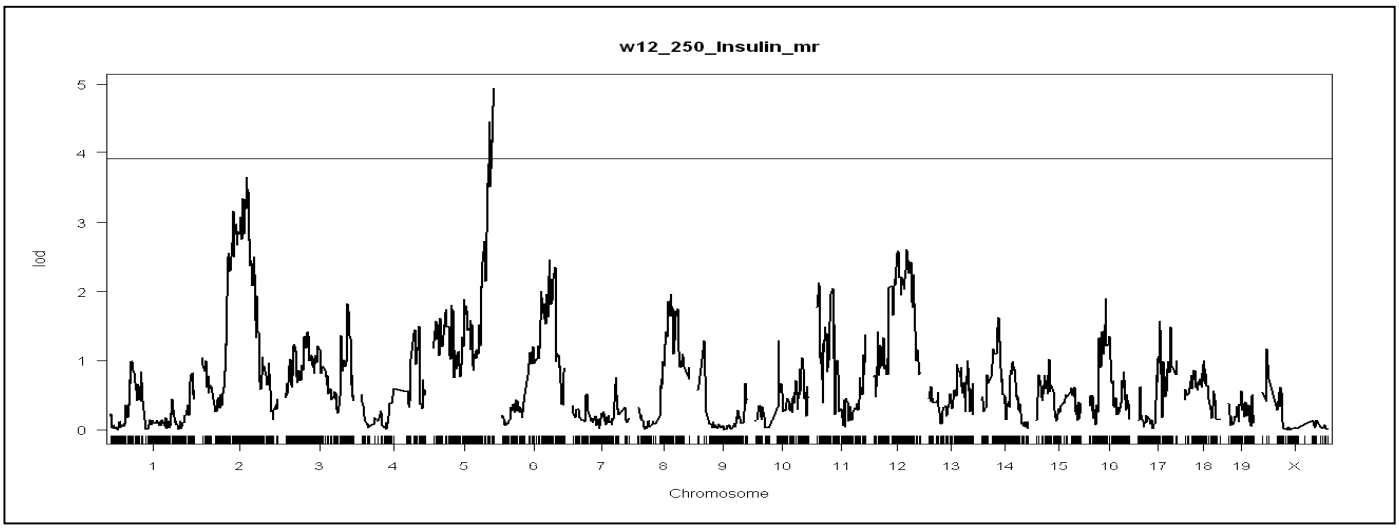
Progress to date:

Tissues and basic phenotype data have been collected on all animals in both the 4-5 week group and in the 12-week group. Plasma samples have been analyzed for lipids (total cholesterol, HDL cholesterol, free cholesterol, triglycerides and free fatty acids) glucose, insulin and a panel of approximately 50 plasma cytokines. For this project, we have recently completed analysis of blood urea nitrogen and this will be followed by analysis for creatinine and albumin.

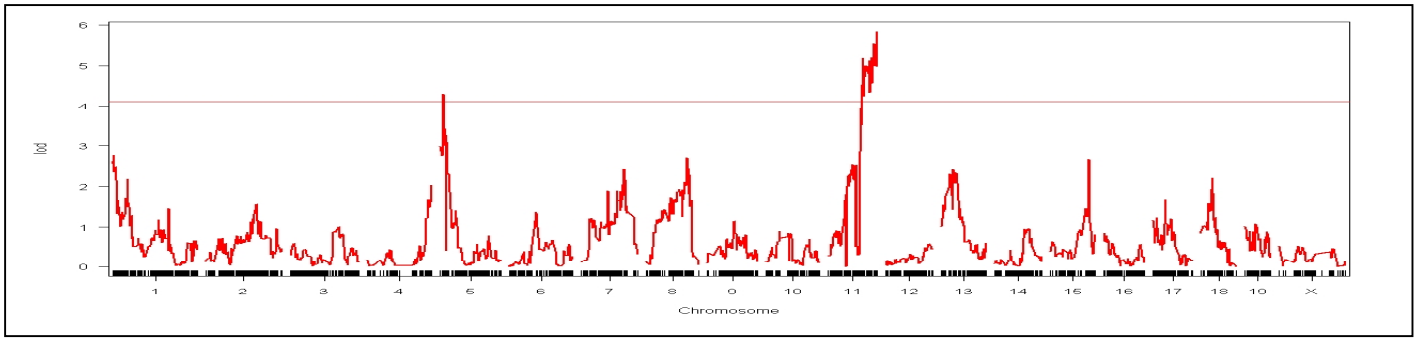
Lipid accumulation in the liver has been measured in the liver of all animals by NMR analysis.

Genomic DNA has been isolated and analyzed for a panel of 5000 SNP markers of which, about 2500 are polymorphic between the parental strains DBA/2 and C57BL/6J. This genotype data is being used for mapping of gene expression eQTLs and of diabetes and complication-related phenotypes.

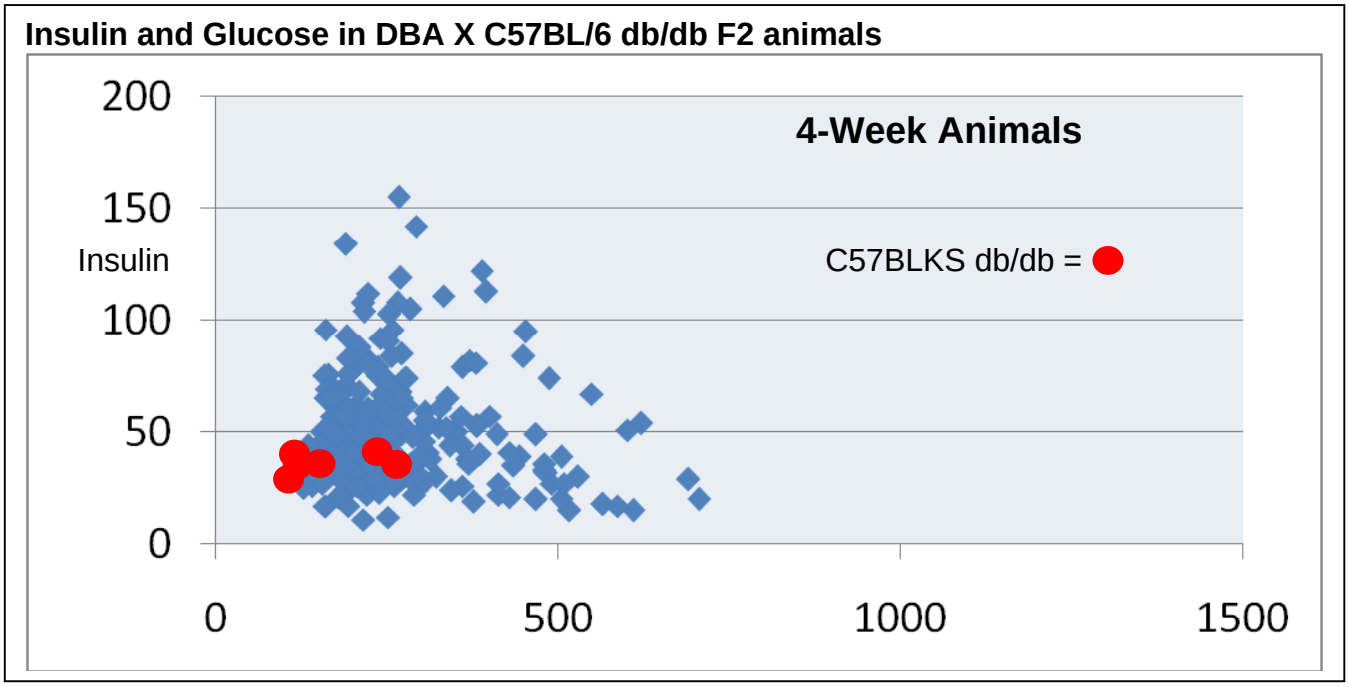


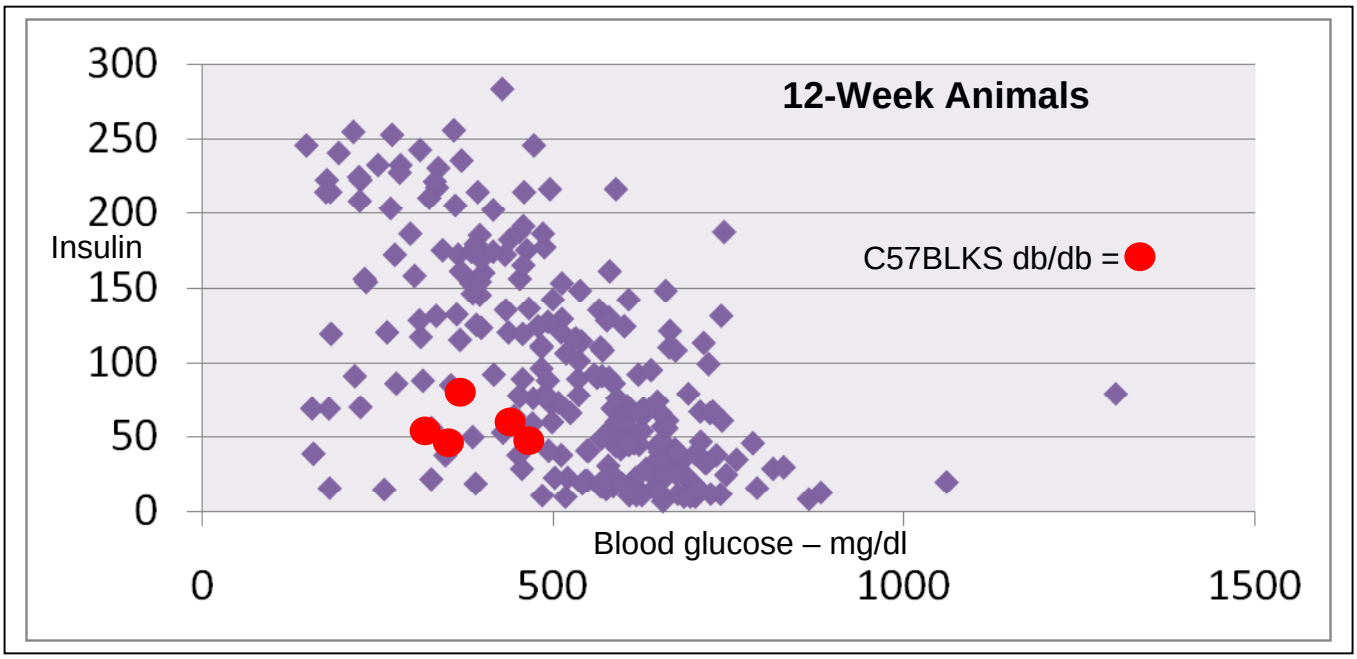


Mapping of diabetes related phenotypes: Plasma insulin levels at 4 weeks (top) and 12 weeks. There is a significant QTL for insulin on Chr 5 at both ages. The horizontal lines indicate genome-wide significance based on permutation tests.



Mapping of diabetes related phenotypes: Percent body fat measured by NMR shows significant QTLs on Chr 5 and Chr 11 in female mice. For male mice, the Chr 5 QTL is absent.

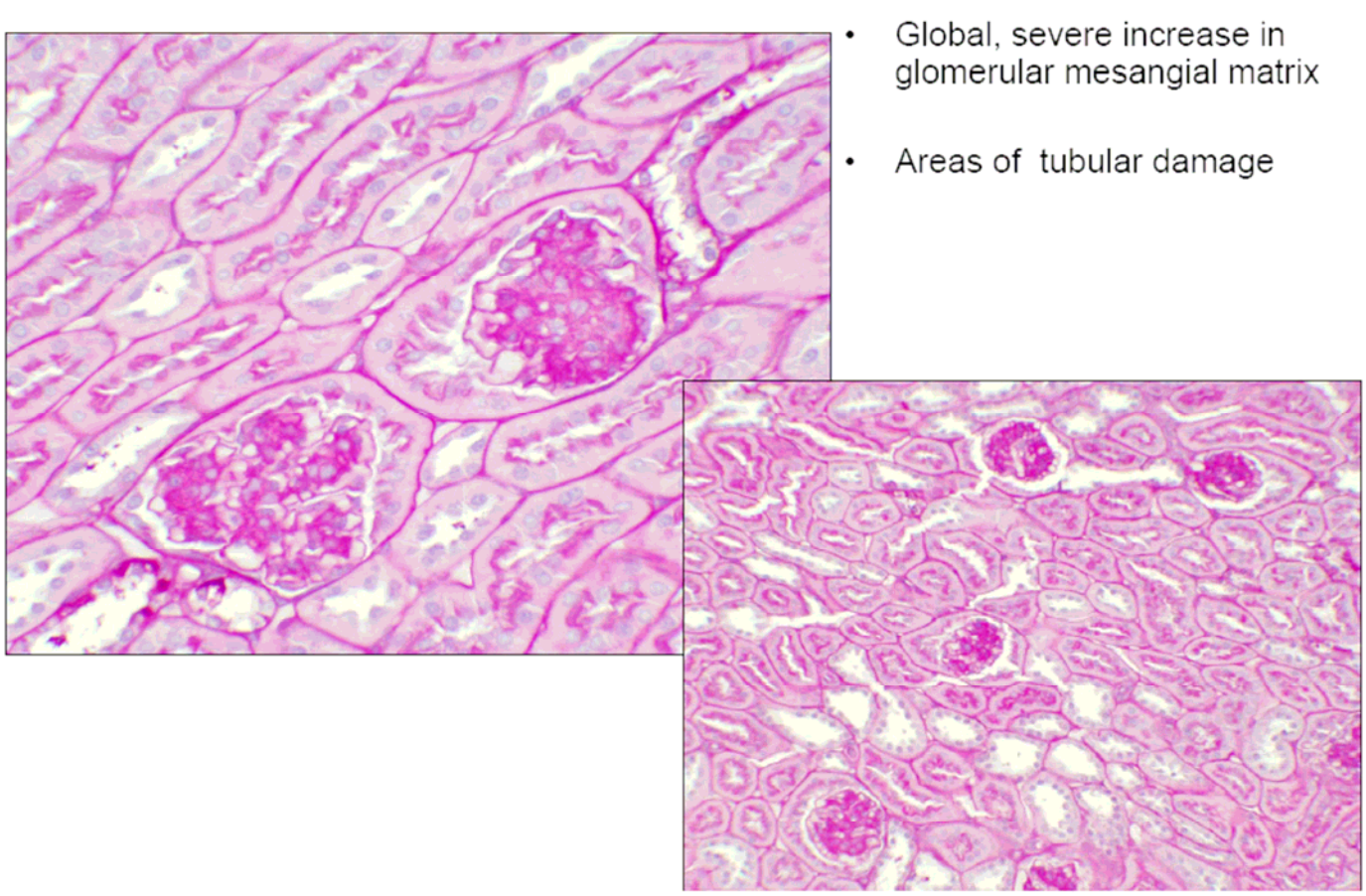




At four weeks of age, most F2 db/db animals have low plasma glucose and insulin levels, similar to C57BLKS db/db mice. Surprisingly, some F2 animals showed elevated insulin and/or glucose levels indicative of more severe insulin resistance/prediabetes than C57BLKS db/db. With age, this trend came to predominate with most F2 animals showing insulin resistance (high insulin, low glucose) or diabetes (low insulin, high glucose) or elevated insulin and glucose suggestive of a transition between insulin resistance and diabetes. Surprisingly, this progression was generally more severe in F2 animals than C57BLKS db/db.

Expression analysis has been completed in four tissues: liver, adipose, pancreatic islets and duodenum. Additional tissues are currently being processed.

Initial samples from the collection of 100 hearts and kidneys have analyzed by the MMPC in Seattle. Histological assessment of these samples has been very promising for the kidneys, with samples from high glucose F2 animals showing more extreme nephropathic histology than C57BLKS db/db mice or any other common mouse model.



Kidney histology from a high-glucose DBA X C57BL/6 db/db F2 animal (Seattle MMPC)

Unfortunately, initial assessment of the heart histology has not proven to be as fruitful, possibly the result of a problem with tissue fixation. We are now exploring alternate histological approaches for heart and working to complete the kidney histology.

The next few months will be used to complete the histology and expression analysis for use in constructing gene networks correlating with diabetic and complication-related phenotypes.