Animal Models of Diabetic Complications Consortium
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"Atherosclerosis and other complications in the hyperlipidemic BKS diabetic mouse"

University of California, Los Angeles

Principal Investigator
Richard C. Davis

Address: **UCLA Department of Medicine**
Cardiology Division
47-123 Center for Health Sciences
Los Angeles, CA 90095-1679

Phone: **(310) 206-4758**
FAX: **(310) 825-2450**

E-mail: **davisr@ucla.edu**

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Part A:

Principal Investigator's Summary

1. **Program Accomplishments:**

Mapping genetic loci that impact diabetic complications.
In prior years, we have examined differences in gene expression profiles between the C57BL/6 and C57BLKS in the context of leptin-receptor deficiency. Lack of the leptin receptor leads to hyperphagia and extreme obesity in both strains but the C57BLKS strain is much more susceptible to diabetes and its complications including cardiomyopathy, nephropathy and neuropathy. We hypothesize that that multiple genetic differences between C57BL/6 and C57BLKS contribute to these differences in susceptibility and that identifying the underlying pathways will help in understanding the genetics of predisposition to diabetes and its complications in humans.

As part of an independently funded project, we have carried out a cross between DBA/2 and C57BL/6 carrying a defective leptin receptor. In previous work, we showed that C57BLKS is a genetic mixture of C57BL/6 and DBA with a small contribution from an unidentified strain. Moreover, there is evidence that the diabetes susceptibility observed in C57BLKS arises from the regions of genome inherited from DBA. Thus, we expect that the F2 cross between C57BL/6 and DBA will reveal map locations for genes important to diabetes susceptibility in C57BLKS as well as additional loci from DBA genomic regions that are not incorporated in C57BLKS.

The basic focus of the cross is to map genetic loci that contribute to the vast differences in diabetes susceptibility seen between C57BL/6 and DBA and between C57BL/6 and C57BLKS. However, for this project, we are also analyzing differences in diabetic complications. In particular, we collected heart, kidney and paw-pads from animals at 12 weeks of age after onset of hyperglycemia. From the same animals, we have collected plasma and urine.

For the basic cross, we have genotype data for approximately 2000 informative SNPs between C57BL/6 and DBA. We are using that data to map quantitative trait loci (QTLs) for diabetes related traits such as adiposity, plasma glucose and insulin. The same genotype information will be used for mapping complication/related phenotypes for the present study. We are now completing analysis of BUN and will measure glucose, albumin and creatinine in the urine. Under funding from an AMDCC pilot and feasibility grant we have forwarded heart and kidney tissues from a subset of these mice to Renee LeBoeuf at the University of Washington MMPC for histological analysis of traits related to diabetic cardiomyopathy and nephropathy. We will then use the genotype data to identify QTLs for these complication-related phenotypes.

Impact of 5-lipoxygenase (5LO) on Pancreatic Function

C57BL/6 mice carrying a knockout of 5LO develop marked insulin resistance. To test the impact of this transgene on beta cell function, we have compared transgenic and control mice for islet response to glucose stimulus. Like C57BLKS islets, islets from the 5LO knockout mouse show reduced insulin secretion, reduced beta-cell proliferation and increased islet apoptosis in response to glucose stimulation. We hypothesize that the similarity between C57BLKS and 5LO knockout mice derives from reduced functionality of the 5LO gene carried by C57BLKS. Because the 5LO gene of C57BLKS derives from DBA, we tested islet function in congenic mice carrying the DBA allele for 5LO on a C57BL/6 background. In one such test, we observed a similar reduced insulin secretion, reduced beta-cell proliferation and increased islet apoptosis in response to glucose stimulation. Replication and extension of these experiments is underway.

Impact of 5LO transgenic and knockout mice on atherosclerosis in db/db ApoE-/- mice

We have completed construction of mice carrying the transgene or knockout for 5LO on the C57BL/6 db/db ApoE-/- background.

C57BL/6 carrying the db mutation and 5LO transgene or knockout have been bred and we are breeding to establish the ApoE knockout on the same background.

Characterization of impact of these congenic regions on atherosclerosis and diabetes susceptibility compared to C57BL6 Apo E -/- mice is ongoing.

Differential diabetes susceptibility between C57BLKS and C57BL/6.

Analysis of genome wide expression differences between C57BLKS db/db and C57BL/6 db/db mice in muscle, liver and adipose tissue was used to Identify sets of differentially expressed genes in each tissue and enrichment of these genes in relevant metabolic pathways. Significantly, in livers of 4 week-old C57BLKS db/db mice, we observed reduced expression of most genes involved in triglyceride synthesis and lipogenesis. This observation was consistent with direct measures of decreased hepatic lipogenesis and TG synthesis. At the same time C57BLKS db/db mice show decreased expression of genes involved in glucose utilization, and impaired suppression of gluconeogenic genes. These differences occur prior to the onset of beta-cell failure and diabetes suggesting that they may contribute to this process. Recent euglycemic clamp studies now show that dramatic differences in hepatic insulin resistance precede the development of diabetes in these animals and may partially underlie the differential susceptibility. (Davis, et al., submitted).

2. Collaboration:

As part of the C57BL/6 X DBA db/db F2 cross, we have collected footpads for histological evaluation for neuropathy at U. of Michigan. In addition, histological evaluation of kidneys and hearts from the cross from the cross are in progress at the Seattle MMPC with support from the pilot and feasibility award. Finally, we have a strong ongoing collaboration with Miklos Peterfy at Cedars Sinai. As part of this work, he used NMR to evaluate livers from the F2 cross for steatosis associated with obesity and diabetes. In addition, we have an active collaboration with Dr. Peterfy to assess the basis for tissue-specific differences in insulin sensitivity between C57BLKSdb/db and C57Bl/6db/db mice.

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3. Address previous EAC comments:

Comment:……Potentially exciting is the use of gene expression analysis to identify gene modules that are coordinately regulated at the tissue level that may help explain disease phenotypes. With proper validation, these modules aid in the understanding of disease regulation as well as serve as therapeutic targets. Importantly, the accurate assessment of phenotype is important for these correlations to be valuable. Thus, equal care should be given to phenotype quantitation as is being given to gene expression analysis. Obviously the collaboration with Dr. Horvath is considered a strength in these endeavors. Further collaboration with the U of Michigan group is also encouraged.

Response: As part of this work we have included as many relevant phenotypic characterizations as feasible. To date, we have evaluated approximately 70 quantitative phenotypes. In addition to the obvious measures of bodyweight, adiposity, plasma glucose and insulin, we have measured plasma lipids (triglycerides, total cholesterol, HDL cholesterol, unesterified cholesterol and free fatty acid), various organ weights including weights in various fat depots, fat accumulation in the liver and a panel of cytokines in plasma. Histological and biochemical measures of nephropathy and cardiomyopathy are underway. We have collected footpads for evaluation at U. of Michigan.

Comment:……Differences between C57BLKSdb/db and C57Bl/6db/db in their susceptibility to beta cell failure may be very insightful for understanding the development of diabetes. As above, gene expression profiles may help identify gene networks that regulate beta cell function and failure.

Response: Groups of C57BLKSdb/db and C57Bl/6db/db mice were collected in parallel to mice in the F2 cross for comparison of biochemical, phenotypic and gene expression profiles. This includes beta cell expression profiles.

Comment:……Experiments with the 5-LO mice to study the effects on pancreatic function are intriguing and need to be repeated.

Response: Repeats of these experiments are underway.

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4. Publications:

Davis, R. C., Castellani, L. W., Hosseini, M., Ben-Zeev, O., Mao, H. Z., Weinstein, M. M., Kim, J., Lusis, A. J., and Peterfy, M. (2009) Early hepatic insulin resistance precedes the onset of diabetes in obese C57BLKS-db/db mice. Submitted