Animal Models of Diabetic Complications Consortium

(U01 HL087944)

Annual Report
(2008)

"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**

Table of Contents

Animal Models of Diabetic Complications Consortium
(U01 HL087944)

Part A:

Principal Investigator's Summary

1. Program Accomplishments:

1) C57BLKS db/db ApoE-/- mice as a model of atherosclerosis in diabetes
Breeding of this strain has been completed and we are now testing this model strain in comparison to control C57BL/6 db/db ApoE-/- mice for atherosclerotic lesion development and associated gene expression differences in relevant tissues.

2) Pancreatic function in C57BLKS**.**
C57BLKS db/db mice develop hyperglycemia and apparent beta cell failure whereas C57BL/6 db/db mice develop hyperinsulinemia but maintain relatively normal glucose regulation. :
To test if this results from inherent susceptibility to beta cell failure in C57BLKS, we previously reported initial comparisons of C57BLKS with C57BL/6 for sensitivity to hyperglycemia-induced suppression of glucose stimulated insulin secretion in isolated pancreatic islets. These follow-up studies confirm reduced insulin secretion, reduced beta-cell proliferation and increased islet apoptosis in response to glucose stimulation. We are now profiling gene expression differences between pancreatic islets of young C57BLKS db/db and C57BL/6 db/db mice. These expression differences will be evaluated in light of pancreatic islet gene networks that we are identifying in F2 (C57BL/6 X DBA/2) db/db mice.
.
3) 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. However, a repeat of that experiment failed and we are now breeding animals for a third test.

4) Construction of 5LO transgenic and knockout mice on C57BL/6 db/db

ApoE-/- background.

As described in grant proposal we have been completing 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.
This year we began characterization of impact of these congenic regions on atherosclerosis and diabetes susceptibility compared to C57BL6 Apo E -/- mice.

5) Gene Expression Profile of C57BLKS
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. (Davis, et al., submitted).

2. Collaboration:

As part of an independently funded project, we are carrying out a cross between DBA/2 and C57BL/6 carrying a defective leptin receptor. The focus of this work is to identify genes and pathways contributing to diabetes susceptibility. However, we are also collecting tissues relevant to diabetic complications.

In particular, we are collecting heart and kidney and are submitting an application for Pilot and Feasibility support monies to identify the underlying genes for cardiomyopathy and nephropathy. In this grant, we propose to perform expression array profiling of the kidneys and hearts in a subset of these mice and identify transcripts whose levels correlate with clinical phenotypes (tissue histology, excreted albumin and creatinine, blood urea nitrogen, plasma creatinine, obesity, insulin levels, lipoprotein levels, blood pressure) and whose levels are regulated by quantitative trait loci (QTL) for nephropathy and cardiomyopathy. We hypothesize that we will identify genes whose expression correlates strongly with the clinical traits and whose levels are determined by clinical trait.

In collaboration with Steve Horvath of Biomathematics and Human Genetics at UCLA, we have recently constructed co-expression networks for metabolic and

cardiovascular traits and have identified network “modules” that correlate strongly with clinical traits such as inflammation, adiposity and insulin levels. With funding from the pilot and feasibility grant, we will apply this strategy to construct heart and kidney networks and relate these to pathology. We hypothesize that we will identify gene networks strongly correlated with nephropathy and cardiomyopathy that will help identify pathologic pathways and mechanisms.

From this same cross, we are collecting paw-pads as a resource to pursue QTL mapping of diabetic neuropathy. We are collaborating with Eva Feldman within the AMDCC to histologically assess these footpads. While, we will not assess gene expression in any nerve tissue from these animals, our intent is to use histological data for neuropathy to identify QTL for this trait.

3. Address previous EAC comments:

Should future AMDCC efforts be more heavily weighted toward gene discovery (or validation of human genetic findings)?

How can the information on the genetic architecture of complex traits and the discovery of genetic variants for types 1 and 2 diabetes be incorporated into AMDCC plans and milestones?

I think that the gene network approach described above goes a long way toward overcoming the difficulties encountered with the single gene approach that we have been pursuing. Even if we combine several single-gene modifications by breeding, this approach rapidly becomes impracticable with increasing numbers of genes. At the same time, while single- or oligo-gene modifications may force the mouse to be more “human-like” and may replicate aspects of the disease phenotypes, it usually fails to reveal much additional about the other pathways that underlie an inherently multigenic disease. Perhaps more seriously, knockouts and transgenics force an extreme perturbation in a single biochemical reaction unlike the situation in most human incidence of the disease where small perturbations of many genes are assumed to combine in generating a susceptible individual. The fact that the disease phenotype is produced by such an unnatural distortion of a single pathway raises questions about artificiality in the model itself and calls into question the applicability of such a model in testing potential therapies.

By contrast, the strategy of identifying gene networks associated with diabetes and its complications has several appealing features. First, network analysis, obtained by comparing expression levels among many genetically varying individuals has shown that groups of genes are co-regulated, in that, detected mRNA levels within the gene-group are highly correlated and are coordinately increased or decreased in response to genetic and environmental perturbations. Moreover, these coordinately regulated gene-groups, or “co-expression modules” can be associated with specific metabolic pathways, tissue functions or disease states. Unlike single gene QTL’s, the correlation of these network modules with disease phenotypes is quite strong so that we see modules accounting for a high % of a complex phenotype (R-squared = 0.1 to 0.4 or higher.)

Should efforts directed at mapping QTLs associated with diabetic complications be expanded under the AMDCC?

Yes, if they are gene network QTL's.

Should additional efforts be made to "tap" this (Matthias Kretzler's dataset) resource as a way to identify candidate genes for AMDCC study?

Yes, because network modules are often conserved across species, modules developed in mice might be a good tool screen for relevant differentially expressed genes in Kretzler's dataset .

Is the value of outbred mouse lines being fully utilized for the study of diabetic complications?

I think there will be more opportunity now, especially as high-density genotyping becomes more available. Alternatively, strain surveys where dense genotype information is known or could be deduced might be applied in the near future.

4. Davis

a. Interesting strain differences in STZ susceptibility.

As suggested above, I think that applying STZ in a strain survey where dense genotype information is known or could be deduced might be applied in the near future. We are doing preliminary work with strain surveys at the present time and have some promising mapping results for complex traits. If this result holds up, I would definitely suggest a similar approach with STZ induced diabetes.

b. There is continued progress towards identifying causes of the BKS diabetes susceptibility. The proposed model BKS-db/db ApoE-/- may be problematic because; how closely does it resemble a human lipid profile? Can the B6/BKS QTL help there? These animals should provide insight as to the impact of 5-LO.

Yes, the cross described above should go a long way towards identifying causes of BKS diabetes susceptibility. ApoE-/- may be problematic as a single gene perturbation for the reason suggested, but in the context of a cross or strain survey may be suitable as a perturbation for revealing atherosclerosis-related modules. Yes, the cross should be quite revealing about 5LO.

c. While it is obviously interesting to look at gene expression in muscle, adipose, and liver tissue for the etiology of insulin resistance, it would also be informative to look at other tissues related to diabetic complications (e.g., heart, vascular, kidney).

I agree, and the proposed work in our Pilot and Feasibility grant application takes exactly this approach.

4. Publications:

• Peterfy, M., Davis, R.C., Lusis, A.J. (2007) Metabolic syndrome as a modifier of atherosclerosis in murine models. Curr. Drug Targets 8: 1215-20 PMID: 18045100

• Davis, R.C., Jin, A., Rosales, M., Yu, S., Xia, X.-Y., Ranola, K., Schadt, E.E., Lusis, A.J. (2007) A genome-wide set of congenic mouse strains derived from CAST/Ei on a C57BL/6 background, Genomics 90:306-13.

• Davis, R.C., Castellani, L., Ben-Zeev, O., Mao, H.Z., Weinstein, M.M., Lusis, A.J., Péterfy, M. (2008) Impaired hepatic lipogenic capacity precedes the onset of diabetes in obese C57BLKS-db/db mice, (Submitted)