Animal Models of Diabetic Complications Consortium
(U01 HL087944)

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"Atherosclerosis and other complications in the hyperlipidemic BKS diabetic mouse"

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Part A:

Principal Investigator's Summary

1. Program Accomplishments:

We are using a variety of genomic and genetic approaches to identify genes and pathways that lead to increased susceptibility to diabetes and its complications in the BKS mouse. In particular, we will evaluate the role of 5LO and other candidate genes in this susceptibility. The results will be useful in developing diagnostic, therapeutic or preventative measures for these maladies.

Studies, Results and Plans

1) C57BLKS db/db ApoE-/- mice as a model of atherosclerosis in diabetes

We are finalizing development of this strain using the initial stocks that we began breeding at UCLA as well as the strain developed by Matt Dwyer. The strain from Dr. Dwyer is further along (N10) but still requires breeding to establish a homozygous ApoE knockout stock carrying the db mutation.

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 compared C57BLKS with C57BL/6 for sensitivity to hyperglycemia-induced suppression of glucose stimulated insulin secretion in isolated pancreatic islets. Initial results from these experiments showed reduced insulin secretion, reduced beta-cell proliferation and increased islet apoptosis in response to glucose stimulation. To further identify the impact of genetic differences between C57BLKS and C57BL/6 on gene expression networks in pancreas we will analyze gene expression profiles in isolated pancreatic islets.

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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 educed 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 will now test islet function in congenic mice carrying the DBA allele for 5LO on a C57BL/6 background.

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.

We will now begin characterization of impact of these congenic regions on atherosclerosis and diabetes susceptibility compared to C57BL6 Apo E -/- mice..

5) Gene Expression Profile of C57BLKS

As described in the grant application, we have used high density SNP mapping to identify the regions of the C57BLKS genome that originate from DBA and, there is evidence that the increased diabetes susceptibility of C57BLKS derives specifically from these regions. Further, we have used expression analysis of an F2 cross between C57BL/6 and DBA to characterize modules of genes in co-expression networks and to identify cis-acting QTL between these two strains that likely underlie the perturbations of these expression modules. We have now applied these data to the analysis of genome wide expression differences between C57BLKS db/db and C57BL/6 db/db mice in muscle, liver and adipose tissue. We will:

a) Identify sets of differentially expressed genes in each tissue and enrichment of these genes in relevant metabolic pathways.

b) Identify co-expression network modules most impacted by the regions of genetic contamination in C57BLKS

c) Identify loci in C57BLKS that appear to modulate gene expression shifts related to diabetes and insulin resistance

As described in the grant application, we have previously constructed a genome wide library of congenic strains carrying DBA sequences introgressed onto a C57BL/6 background. We will use the above expression analysis to assist in selecting congenics to be bred to the C57BL/6 db/db background to test these congenic loci for their impact on diabetes susceptibility.

2. Collaboration:

C57BLKS db/db ApoE-/- is a primary model of atherosclerosis in diabetes in this proposal. We are finalizing development of this strain using the initial stocks that we began breeding at UCLA as well as the strain developed by Matt Dwyer. The strain from Dr. Dwyer is further along (N10) but still requires breeding to establish a homozygous ApoE knockout stock carrying the db mutation here at UCLA. That breeding is underway.

3. Address previous EAC comments:

N/A

4. Publications:

•Estrada-Smith, D., Collins, A. R., Wang, X. P., Crockett, C., Castellani, L. W., Lusis, A. J., and Davis, R. C. (2006) Impact of chromosome 2 obesity loci on cardiovascular complications of insulin resistance in LDL receptor-deficient C57BL/6 mice. Diabetes, 55:2265-71

- Hsueh, W.A., Abel, E.D., Breslow, J.L., Maeda, N., Davis, R.C., Fisher, E.A., Dansky, H., McClain, D.A., McIndoe, R., Wassef, M.K., Babadan-Diehl, C., Goldberg, I.J. (2007) Recipes for creating animal models of diabetic cardiovascular disease. Circ Res, 100:1415-27

- 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 (Epub ahead of print: PMID: 17600671).