Animal Models of Diabetic Complications Consortium
(U01 DK076131)

Annual Report
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“Bradykinin, nitric oxide and mitochondrial damage in diabetic complications**”**

UNC

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Part A:

Principal Investigator's Summary

1. Program Accomplishments:

Hypothesis

Our long term objective is to unravel whether oxidative stress is required for the development and progression of diabetic nephropathy, and to determine the role of mitochondrial mutations in diabetic complications.

Progress toward stated milestones

A. Model development and physiological assessment

- Phenotyping completed of diabetic nephropathy in whole body B1 and B2 receptor double null Akita diabetic mice compared with B2 null Akita mice (manuscript in preparation).
- Investigation of the role of coagulation and increased tissue factor (coagulation factor III) in exacerbation of diabetic nephropathy in eNOS null STZ diabetic mice fed normal chow and a high fat diet (manuscript submitted).
- Generation of F1(C57BL/6 x129SvEV) eNOS-/- Akita/+ mice, eNOS+/- Akita/+ mice and eNOS+/+ Akita/+ mice, and detailed phenotyping of their diabetic nephropathy.
- Detailed investigation of the mechanism of why mitochondrial DNA polymerase gamma *Polg* mutant (D257A, a proof reading defect) Akita/+ mice have less severe diabetes relative to Akita/+ mice with wild type *Polg* (manuscript in preparation).

B. Production of mice with mutations in candidate susceptibility genes for diabetic complications

- Production of mice allowing conditional deletion of B1 B2 receptors: Chimeras at Jackson Lab. Germline transmission awaited. Generation of new targeted ES cells and blastocyst injections continuing.
- Production of mice with conditional D257A *Polg*: Chimeras at UNC. Germline transmission awaited. Generation of new targeted ES cells and blastocyst injections continuing.

Plans for the Upcoming Year

Because our projects are progressing very well, we do not anticipate any changes in our plans.

- To determine the effects on diabetic complications of eliminating both bradykinin receptors in a tissue or cell specific manner, and the effects of reducing oxidative stress in these mice.
- To investigate the role of tissue factor in the development of diabetic complications in eNOS null mice.
- To determine the effects on diabetic complications of homozygosity of D257A mutation of *Polg* in a tissue or cell specific manner.

Preliminary Milestones for 2010 and Beyond

- Mice lacking both B1 and B2 receptors are expected to prove interesting models of diabetic complications in addition to nephropathy.
- Heterozygous eNOS+/- Akita/+ mice show diabetic nephropathy similar in severity to that of homozygous eNOS-/-Akita/+ mice, and should prove to be an excellent model of diabetic complications.
- Coagulation and inflammation are likely responsible for exacerbating diabetic complications in the eNOS+/- (or-/-) Akita/+ mice even in the absence of further obvious increases in oxidative stress.
- Increasing the frequency of mtDNA mutations by introducing a proof reading defect into *Polg* ameliorate diabetes, and thus its complications. Our current evidence indicate that this is likely due to a decrease in food absorption in the intestine caused by increased apoptosis of intestinal cells and/or suppression of appetite due to a decrease in some unknown factor(s) from the testis, which also has apoptosis and shows scarcity of cells.
- We have started and will continue transferring primary data to the AMDCC database.

2. Collaboration:

With other AMDCC PIs

- Diabetic mice lacking both the bradykinin B1 and B2 receptor genes have been sent to Drs. Eva Feldman, Philip Tsao, and Dale Abel for studies of diabetic neuropathy and cardiomyopathy.
- F1 (C57BL/6 x 129SvEv) heterozygous eNOS+/- Akita mice will be sent to Drs. Eva Feldman and Dale Abel for diabetic neuropathy and cardiomyopathy studies.
- Dr. Moshe Levi has found that the Farnesoid X Receptor regulates B2 receptor expression in the kidney, and will investigate whether the effect of FXR on diabetic nephropathy is mediated by B2R by testing the effects of an FXR agonist on B2R null Akita mice.

With Jax

- Bradykinin B2 receptor-/- Akita/+ mice: sent to Jax, and being backcrossed to C57BL/6, and to the 129 SvEv and DBA/2J backgrounds.
- B1RB2R -/- mice (made in C57BL/6 ES cells and so pure C57BL/6): sent to Jax, being phenotyped and maintained on C57BL/6, and backcrossed to 129 SvEv and DBA/2J for distribution to other members of the consortium.
- Male eNOS-/- (pure 129 SvEv) and female eNOS+/- Akita/+ (pure B6): sent to Jax, F1 eNOS+/- Akita mice are being generated for use by the consortium and are being phenotyped.

With the MMPCs N/A

With other non-AMDCC PIs N/A

3. Address previous EAC comments:

EAC comments: Good progress on both general aims, role of bradykinin and mitochondrial mutation in generation and/or modulation of the DN phenotype. Mice should be phenotyped for other vascular abnormalities (endothelial function, atherosclerosis) in addition to the ongoing myocardial studies. It would also be interesting to perform a whole battery of CV phenotyping with the bradykinin receptor double knockouts given the relationship between bradykinin and the renin-angiotensin system.

Response: Determination of atherosclerosis in B1B2 double knockout mice is in progress; studies of the neural and cardiac complications of the mice are well advanced. Cardiac and neural phenotyping of F1 heterozygous eNOS+/- Akita mice is planned.

4. Publications:

Kakoki M, Smithies O. The kallikrein-kinin system in health and in diseases of the kidney. Kidney Int. 2009 75(10):1019-30. PMID: 19190676

Li F, Wang C-H, Thai T, Boysen G, Xu L, Turner A, Wolberg AS, Mackman N, Maeda N, Takahashi N. Hypercoagulability and elevated tissue factor exacerbate diabetic nephropathy in mice lacking eNOS J Thromb Haemat (submitted)

Brosius FCIII, Alpers C, Bottinger E, Breyer MD, Coffman TM, Kretzler M, Gurley SB, Harris RC, Kakoki M, Leiter EH, Levi M, Quaggin SE, Sharma K, Smithies O, Susztak K,Takahashi N. Update on Mouse Models of Diabetic Nephropathy: A Midstream Analysis from the Animal Models of Diabetic Complications Consortium J Am Soc Nephrol (submitted).

Li F, Arbones-Mainar JM, Maeda N, Takahashi N. Nitric Oxide increases adiponectin through a guanylyl cyclase-dependent pathway. (in preparation)