AMDCC Annual Report (2011)

PI: BOTTINGER, ERWIN P
Project Title: Role and Mechanisms of Epithelial Injury in Diabetic Nephropathy
Grant Number: U01 DK060995

Abstract: Glucotoxicity, lipotoxicity, advanced glycation end products (AGE), and reactive oxygen species (ROS) have emerged as important mediators of diabetes-induced tissue injury. However, the precise cellular targets and molecular mechanisms by which these mediators cause cellular injury in the kidney remain poorly defined. Both, glomerular (podocytes) and tubular epithelial cells are now emerging as important targets for diabetes-induced injury in diabetic nephropathy (DN). Thus, podocyte depletion has been described recently in humans with type 1 (T1DM) and type 2 (T2DM) diabetes, and is considered a strong predictor for the development of proteinuria. In addition, tubular epithelial apoptosis and epithelial-mesenchymal transition (EMT) may underlie the initiation of tubulointerstitial progression of DN. We present preliminary results that provide a compelling rationale to focus our research program on investigating the emerging role and mechanisms of epithelial cell injury in DN. Specifically, we will test two novel hypotheses: 1. The peroxisomal membrane proteins Mpv17l and/or Mpv17 are essential regulators of antioxidant defenses in glomerular podocytes and protect against diabetes-induced podocyte apoptosis and podocyte depletion (Bottinger, Mount Sinai); and 2. CD36 scavenger receptor for AGE and/or FFA is an essential mediator of AGE and/or FFA-induced tubular epithelial injury/apoptosis and tubulointerstitial progression of DN (Susztak, AECOM). The Specific Aims of this proposal are to test: 1. whether peroxisomal membrane proteins regulate antioxidant defense mechanisms in podocytes, and protect against ROS-mediated podocyte injury/apoptosis induced by diabetes 2. whether Mpv17-deficiency accelerates and increases podocyte apoptosis and depletion leading to progressive glomerulosclerosis and/or nodules in diabetic mouse models 3. which CD36-dependent intracellular pathways signal AGE and FFA induced tubular epithelial injury/apoptosis 4. whether proximal tubular overexpression of AGE binding proteins CD36 and RAGE leads to increased tubular epithelial injury/apoptosis and tubulointerstitial progression of DN in mice.

The specific aims of the current proposal were to examine:

1. whether mitochondrial membrane proteins Mpv17 and Mpv17l regulate antioxidant defense mechanisms in podocytes and protect against ROS-mediated podocyte injury/apoptosis induced by diabetes
2. whether Mpv17-deficiency accelerates and increases podocyte apoptosis and depletion leading to progressive glomerulosclerosis and/or nodules in diabetic mouse models
3. which CD36-dependent intracellular pathways signal AGE and FFA induced tubular epithelial injury/apoptosis
4. whether proximal tubular overexpression of AGE binding proteins CD36 and RAGE leads to increased tubular epithelial injury/apoptosis and tubulointerstitial progression of DN in mice

Rationale and Relevance

Compelling evidence for important roles of AGE binding protein CD36 and RAGE, and mitochondrial protein Mpv17 in cellular homeostasis exists. CD36 may be a critical mediator of AGE-induced epithelial cell injury. Mitochondrial Mpv17 proteins and mitochondrial antioxidant functions may protect podocytes from ROS-mediated injury and apoptosis. Little is known about the role of AGE and ROS in diabetes-induced epithelial cell injury, including EMT, dedifferentiation, apoptosis, autophagy, or hypertrophy. We propose to explore the roles of AGE-binding proteins and mitochondrial proteins in epithelial cell injury in diabetic nephropathy.

Bottinger Lab

Project 1: "Role Mpv17-family members in DN"

Progress towards stated aims

In vitro studies of Mpv17/Htra2 complexes in mitochondrial function and oxidative stress

Mpv17 studies

Conditionally-immortalized murine podocyte cell lines from Mpv17+/+, +/-, -/- backgrounds
We have conducted bioenergetics studies, including a) ATP content, b) mitochondrial mass (MM), and c) oxygen consumption rate (OCR).
Results: ATP content and OCR were decreased in ko, compared with het and wt podos when cultured under normoglycemic (11 mM Gluc) permissive conditions. High ambient glucose (30 mM) caused further decrease in ATP content and MM when compared to 11 mM Gluc in ko podos (Fig. 1). Interestingly, ATP was increased under low gluc (5 mM) conditions compared with 11 mM Gluc in ko podos (Fig. 1). Interestingly, differentiation induced by shift to non-permissive

conditions (37°C) appeared impaired in het, and even more in ko podos, compared with wt cells, as assessed by reduced expression of podo differentiation marker synaptopodin (not shown).

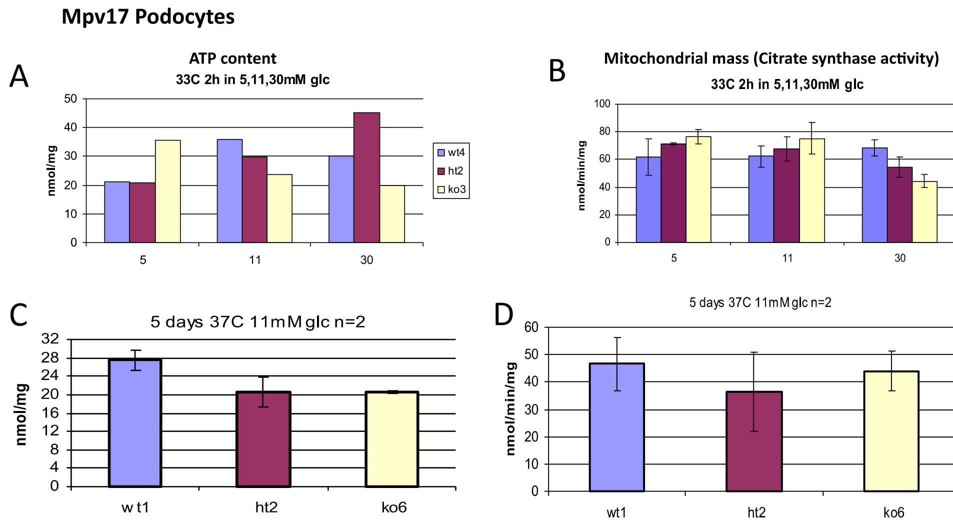


Fig. 1

Oxygen consumption rate (OCR) in glomeruli isolated from Mpv17 wt, het, and ko mice

These in vivo studies confirmed the in vitro results, showing decreased OCR in het and ko glomeruli, when compared with wt gloms (Fig. 2A). Specifically, OCR driven by complex I, but not complex II was decreased in ko gloms compared to wt gloms (Fig 2B).

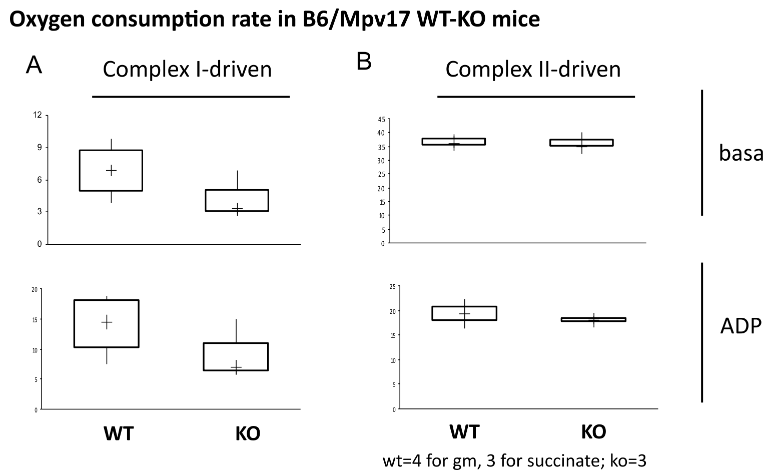


Fig. 2

Bioenergetic studies in Htra2 wt and ko MEFs

We have previously reported that mitochondrial superoxide generation was increased in Htra2 ko compared with wt MEFs (not shown). Using these cells, we conducted additional bioenergetics

studies, including a) ATP content, b) mitochondrial mass (MM), and c) oxygen consumption rate (OCR), and examined OXPHOS protein complexes I – V.

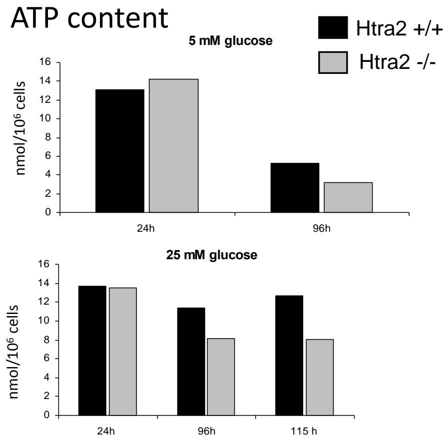


Fig. 3

Under low Gluc (5 mM), basal OCR and MM were increased and ATP content was decreased compared with Htra2 wt MEFs (not shown). Prolonged culture under high gluc conditions (25 mM) for 2 or more day caused decrease in ATP content in ko MEF, but had no effect in wt MEF (Fig. 3).

OXPHOS complexes I and III were increased, and complex IV was decreased in ko MEFs compared with wt MEFs at 5 nM Gluc. High gluc (25 mM) caused reduction of complexes I and III and increase in complex IV in wt MEFs. In ko MEFs 25 mM Gluc reduced complex III, but not complex I (not shown).

Conclusions

- Loss of Mpv17 is associated with reduced mitochondrial respiration and ATP content and impaired differentiation capacity in podocytes, which is further impaired under high ambient glucose conditions.
- OXPHOS complex analysis points to a defect in complex I driven, but not complex II driven mitochondrial respiration.
- Similar results where obtained in Htra2 ko MEFs compared with control MEFs. Thus, loss of Htra2 phenocopies loss of Mpv17, consistent with a functional requirement for Mpv17/Htra2 complex formation.

Plans for the remainder of the year and completion of the project:

- Complete ‘rescue’ experiments, i.e. overexpression of Mpv17 and mutants in wt and ko podos.
- siRNA mediated knockdown of Htra2 in podocytes
- prepare manuscript for publication

Generation of inducible transgenic and knockout models of Mpv17 in mice

Generation and analysis of mice carrying Mpv17 floxed alleles

During the past we collaborated with The JAX lab, who generated high quality chimeras which will be shipped to Mount Sinai during the last week of April. In summary, 8 chimeric mice with >90% Agouti coat color (high percentage) will be shipped.

Plans for the remainder of the year and completion of the project:

- Receive chimeras from JAX lab end of April
- Establish germline transmission and validate Cre recombination at Mpv17 locus
- Establish NPHS2-Cre / Mpv17$^{fl/fl}$ matings for podocyte-selective excision of Mpv17
- Analyze phenotype of non-diabetic podocyte-selective Mpv17-/-
- Establish and analyze STZ-induced T1D model in PodMpv17+/- and PodMpv17-/- mice (anticipated result: acceleration of DN lesions)

Generation of mice carrying Mpv17l floxed exon 3 alleles
We have not succeeded to establish lines with germline transmission, despite exhaustive, even heroic efforts over more than 2 years. We have abandoned this project.

Supplemental ARRA funding progress update

Generation of doxycycline inducible transgenic models for overexpression of Mpv17 in mice and diabetic mouse models

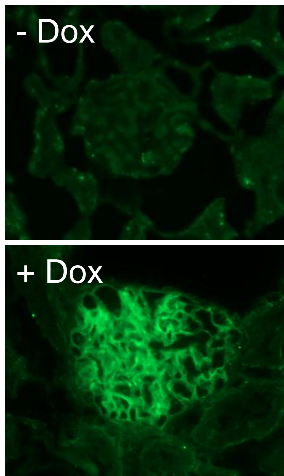


Fig. 4

Generation of Tet-O-Mpv17 transgenic mice
In work supported with supplemental ARRA funding, we generated and validated transgenic mouse model for doxycycline-inducible expression of Mpv17. Tet-O-Mpv17 mice were crossed with NPHS2-rtTA mice (from Dr. Jeffrey Kopp). Doxycycline-inducible expression of Mpv17 in glomerular podocytes in vivo was confirmed by QPCR of Mpv17 mRNA in isolated glomeruli and by anti-Flag tag immunofluorescence staining of mouse kidney sections (Fig. 4).

Future plans:

- Determine effects of shortterm and longterm overexpression of Mpv17 in podocytes on kidney function and structure
- Determine the functional role of Mpv17 expression in podocytes in diabetic models (STZ)

Project 2: "Genetics of glomerular disease susceptibility in murine diabetes models "

Progress towards the stated aims:

Intraglomerular cellular localization of mitochondrial dysfunction and increased oxidative damage.

Our previous analysis was based on studies of whole glomerular extracts, including microarray and bioenergetics studies, suggesting decreased OXPHOS, increased mitochondrial dysfunction. However, while we hypothesized that these strain-specific differences between susceptible

DBA/2J and resistant C57BL/6J had their root cause in direct podocyte injury, this had not been proven experimentally.

Increased mitochondrial ROS generation is particularly damaging to mitochondrial DNA because of the proximity, where it causes oxidative DNA damage that can be assayed by 8-oxo-Guanine labeling. We first applied immunofluorescence labeling for oxidative DNA damage in situ. Glomerular 8-oxo-G staining was detectable only in diabetic D2, but not in B6 mice, confirming that B6 mice were resistant while D2 mice were susceptible to diabetes-induced oxidative DNA damage (Fig. 5A).

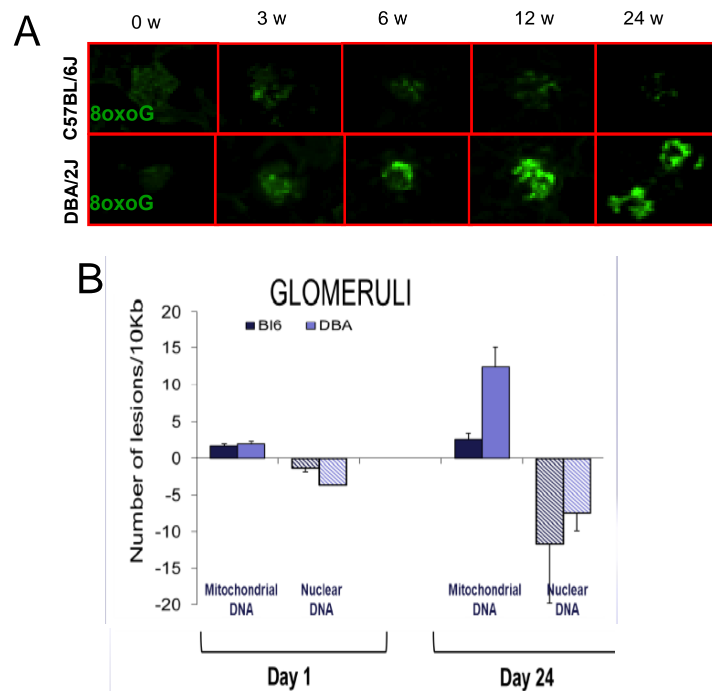


Fig. 5

Next, glomerular DNA was fractionated into nuclear and mitochondrial DNA fractions. Quantitative PCR based analysis allows detection of number of oxidative DNA lesion per 10 kb. On day 1 of STZ-induced hyperglycemia, number of nuclear and mitochondrial DNA lesions was not significantly increased in both, B6 and D2 mice (Fig. 5B). However, after 4 weeks of diabetes, mitochondrial DNA lesions were significantly increased in D2, but not in B6 mice. In contrast nuclear DNA lesions were not significantly increased in either strain (Fig. 5B). These results suggest that oxidative mitochondrial DNA damage is a characteristic of susceptible D2 mice, and absent in resistant B6 mice.

Early preliminary results using double immunofluorescence labeling for 8-oxo-G together with either podocyte marker synaptopodin or endothelial marker Cd31 showed absence of colocalization of 8-oxo-G with synaptopodin, but almost complete colocalization with endothelial Cd31 (not shown) in diabetic D2 mice. These preliminary results suggest that

oxidative DNA damage as a result of OXPHOS and mitochondrial dysfunction may occur predominantly in endothelial cells, but not in podocytes, as we had previously thought.

Conclusions: Our preliminary studies suggest that the cellular target for OXPHOS, mitochondrial dysfunction, oxidative stress, and oxidative DNA damage may be the endothelial compartment, whereas podocytes may not be direct targets of these diabetes-induced cellular defects. Based on these results we revised our working hypothesis: We propose that susceptibility to diabetes-induced glomerulopathy in D2 mice is mediated by mitochondrial dysfunction in endothelial cells, which may generate endothelial to podocyte signaling to induce podocyte apoptosis and/or depletion.

Plans for the remainder of the year and completion of the project:

- Explore glomerular endothelial 8-oxo-G labeling for use as quantitative trait and mapping of susceptibility loci (QTL) using the BXD RI system. We have tissue from all animals available and this analysis can be completed in 3 to 4 months. If the hypothesis is correct that primary susceptibility injury is endothelial cell mitochondrial dysfunction, then we would expect that the susceptibility loci will overlap with the previously identified loci for 'diabetes-induced podocyte loss' DIPL on chr 13 and chr 17.

DIPL is a heritable quantitative trait mapping to loci on chromosomes 13 and 17

As discussed in the previous section, our recent results suggest that the primary genetic susceptibility to diabetes-induced podocyte loss may be affected by endothelial cell OXPHOS and mitochondrial dysfunction susceptibility in response to diabetes. Under this revised model, we propose that DIPL is a secondary mechanism induced by endothelial cell dependent cell-cell signaling. We have therefore not pursuit the fine-mapping studies for DIPL on chr 13 and chr 17. We want to confirm first whether endothelial oxidative damage susceptibility maps to the same loci or not. If yes, fine mapping of chr 13 and chr 17 loci will be conducted based on 8-oxo-G labeling as phenotypic read-out. If not, we propose that chr 13 and chr 17 contain podocyte-intrinsic DIPL pathways that respond differently to inductive endothelial cell signals.

A. Collaborations

With JAX lab: generation of chimera generating Mpv17 floxed alleles
With other AMDCC Members: Matthew Breyer, Eli Lilly & Co
With other non-AMDCC PIs: Robert Williams, University of Tennessee, Memphis

Project 3: "Role of Cd36 in tubulo-interstitial of DN"
Responsible Investigator: Katalin Susztak

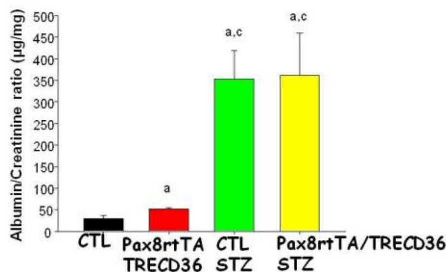
Rationale and Relevance Our preliminary studies showed that as opposed to human diabetic nephropathy the currently examined murine diabetic animal models (db/db and STZ induced diabetes of C57B6J 129SvJ mice) do not develop significant tubulointerstitial fibrosis. We found that expression of a scavenger receptor; CD36 coincided with proximal tubular epithelial cell apoptosis and tubulointerstitial fibrosis in human DNP. CD36 expression was necessary and sufficient to mediate proximal tubular apoptosis induced by glycated albumins and free fatty acid palmitate through sequential activation of src kinase, and proapoptotic p38 MAPK and caspase3. In contrast, paucity of expression of Cd36 in PTEC in diabetic mice with diabetic glomerulopathy was associated with absence of tubular apoptosis and normal tubular epithelium. Mouse PTEC lacked Cd36 were resistant to glycated albumin induced apoptosis. Recombinant expression of CD36 in mouse proximal tubular epithelial cells conferred susceptibility to glycated albumin induced apoptosis. Our findings suggest that CD36 is as essential mediator of proximal tubular apoptosis in human DNP.

Progress towards stated aims:
Since the initially proposed sglt2CD36 transgenic model did not result in tubular transgene expression, and after out setback with MPV virus infection of our animal colony, we are now on track with a new transgenic approach to enable inducible tubular expression of Cd36. For example, we generated a new transgenic line where CD36 is under the tetracycline responsive promoter, using the tet-O-CD36 transgenic construct. We crossed these animals with the Pax8-rtTA animals.

	n	Body Weight	Blood Glucose**
CTL		41.05±4.66	285.80±60.22
Pax8rtTA/ TRECD36		40.63±7.08	325.13±44.34
CTL+STZ		26.33±3.67*	592.86±17.20*
Pax8rtTA/TRE CD36+STZ		27.41±2.76*	590.71±15.92*

Data are presented as mean±SD: * p<0.0001 CTL vs Tg: Blood glucose was measured in the fed state;

Last year we established 2 transgenic lines (Pax8rtTA/TRECD36) where we detected significant mRNA and protein expression of CD36 after doxycycline feeding. These animals are in FvB background. Dox food was initiated at 4 weeks of age and animals were made diabetic with low dose STZ injection. One group of animals were sacrificed at 8 weeks of age and another group was sacrificed at 20 weeks of age. Phenotype analysis was performed at both times. The experiments were complicated by the increased mortality rate of FvB diabetic mice, due polyuria. The body weight and blood glucose values at 20 weeks of age are shown below. Preliminary phenotype analysis at 8 weeks of age indicated mildly but significantly increased tubular epithelial apoptosis rate and increased expression of macrophage marker expression in diabetic Pax8rtTA/TRECD36 mice compared to wild type diabetic mice.



a, p<0.05 *compared to CTL*; c, p<0.05 compared to Tg; Albuminuria was analyzed at 20 weeks old male FvB mice

The preliminary phenotype analysis at 20 weeks of age, showed some increase in albuminuria in the CD36 transgenic mice, however, diabetic control and diabetic CD36 transgenic animals did not show statistically significant differences in urinary albuminuria levels.

Plans for the year and completion of the project:
Complete phenotype analysis, including apoptosis, kidney fibrosis, inflammation renal lipid analysis of Pax8rtTA/TRECD36 animals.
In vitro studies to analyze lipid uptake and metabolism in control and diabetic tubular epithelial cells.
Generate endothelial cell specific CD36 transgenic animals (using the tie2rtTA transgenic mice) and examine diabetic phenotype.
Continue to examine nephropathy phenotype in diabetic FvB mice, including FvB STZ, FvB dbdb and Ove26 FvB mice. We have experienced significant set-back with this project due to increased mortality of diabetic FvB mice at older age (after 18 weeks age). This is mainly due to the polyuria of FvB diabetic mice. We worked with the animal facility and we are using new set of ages with elevated floors. This new method appears to enhance the survival of the animals and minimize the number of animals with urinary tract infections.

Non-AMDCC collaborations:
1, Streamson Chua (AECOM) and Ali Gharavi (Columbia University). Characterization of the nephropathy in FvBdb/db mice. Identification of genes and genetic region responsible for the increased susceptibility of nephropathy in FvB mice.

Address previous EAC comments:

- Has all of your DBA2/J data been uploaded to the website? In particular, documentation of diabetes induced podocyte loss.

Response 1/Bottinger (R1/EB): Our method of podocyte counting is based on two-dimensional analysis using cell-type specific markers. To validate these results, we are currently completing confirmatory, labor-intensive experiments using established methods for estimation of absolute podocyte numbers. Pending the results from confirmatory studies, we have begun to work with Rick McIndoe to create the templates to upload the currently available phenotype data, including our data on podocyte loss during diabetes in susceptible DBA/2J strains

- Has the microarray analysis data of the glomerular transcriptomes been uploaded to the website?

R2/EB: We are completing large scale confirmatory studies using QPCR as an independent method to confirm the key glomerular transcriptome profiles, i.e. running close to 100 QPCR assays. Pending completion of these confirmatory QPCR assays, we have initiated the process of uploading the microarray data with support from Rick McIndoe.

- Has your phenotypic data of the OVE26 mice been uploaded to the website?

R3/KS: Continue to examine nephropathy phenotype in diabetic FvB mice, including FvB STZ, FvB dbdb and Ove26 FvB mice. We have experienced significant set-back with this project due to increased mortality of diabetic FvB mice at older age (after 18 weeks age). This is mainly due to the polyuria of FvB diabetic mice. We worked with the animal facility and we are using new set of ages with elevated floors. This new method appears to enhance the survival of the animals and minimize the number of animals with urinary tract infections.

- After hitting some roadblocks with KO generation, Dr. Bottinger is making good progress. Particularly the genetic studies on podocyte loss look very promising.

R4/EB: Agreed (see also Annual Report)

- Because of problems with establishing diabetes in Mpv17 KO mice, Dr. Bottinger is pursuing a conditional approach. The targeting vector has been generated and passed on to JAX. The project seems to be on track again.

R5/EB: Agreed (see also Annual Report)

- Dr. Bottinger has successfully established immortal podocyte cell lines from the KO mice which should be a useful resource for future studies. Experiments in KO MEFs

have established an increased capacity for mitochondrial superoxide production and plans to investigate mitochondrial function may be informative.

R6/EB: Agreed (see also Annual Report)

- The mapping of podocyte loss alleles in DBA is progressing well. There seems to be an association with mitochondrial function (array results validated by respiration measurements), so this may be a nice tie in with the Mpv17 studies. This array data should be uploaded to the website.

R7/EB: See above response R2/EB

- Dr. Susztak now has tet sensitive CD36 transgenic mice in hand and has begun to characterize them. Dr. Susztak has also begun characterizing various diabetes models on the FVB background for nephropathy and found the FVB-Ove26 most susceptible. It seems this project is back on track.

R8/KS: Agreed (see Annual Report) We are working on phenotyping

- The microarray data that you previously uploaded to the website is a very useful resource. Do you have additional data to upload?

R9/EB: See above response to R2/EB

- Dr. Bottinger continues his systems biology approach. JAX is generating a flox Mpv17l allele. Concern is that effort is too diffuse and progress is slowed. The Mpv17l and podocyte respiratory chain projects seem to focus on a common mechanism, altered mitochondrial respiration. The QTL mapping for podocyte loss represents a second area. In general, QTLs in mice have failed to be informative (i.e. identify the variants). Unless Dr. Bottinger has specific approaches to address the difficulties in fine mapping, he might focus on completion of the other aspects of his projects in the remaining year of funding.

R10/EB: Agreed, we have focused on completing / advancing the other projects and plan to pursue a systematic approach to fine mapping once we will have confirmed the loci for endothelial oxidative DNA damage (i.e. 8-oxo-G labeling) (see Annual Report). See also response R13/EB below.

- After developing new strategies, Dr. Susztak has now generated a transgenic mouse that expressed hCD36 in tubules, testing the hypothesis this molecule is a key regulator/initiator of tubulointerstitial disease in DN. Phenotypic data should be available shortly in STZ-treated double transgenic mice. Presumably she is demonstrating hCD36 is expressed in appropriate subcellular compartment (plasma membrane). The plans for the upcoming year are a little fuzzy. Cell lines from

control and hCD36 transgenic animals have been generated to determine mechanism of CD36 mediated tubular degeneration. No details indicated; experiments may not be pertinent to DN if transgenic animals fail to develop the TI disease. Dr. Susztak also discusses evaluating diabetes models on FVB background, but how the data will guide her experiments (does she want a robust DN phenotype or a mild/absent DN phenotype, which might be better to discern any effect of CD36 expression).

R11/KS: Agreed (see Annual report). Currently we are working on characterization of the transgenic animals. The hypothesis is that TEC CD36 expression will enhance DKD susceptibility, therefore as a first attempt we used STZ injection to induce diabetes. We are also planning to cross CD36 transgenic animals with FvB Akita mice.
In vitro mechanistic studies are planned with tubular epithelial cells isolated from CD36 transgenic animals.

- In the Bottinger lab, studies of Htra2 null MEF cells are underway and suggest increased mitochondrial superoxide production. Generation of Mpv floxed mice are in progress, while Mpv17L has been challenging. The findings from transcriptional studies suggested a loss of oxphos trancripts, related to strains with loss of podocytes. Studies systemic anti-oxidant treatment are planned, as well as heterozygous deletion of complex I in diabetes-resistant mice.

R12/EB: see response R13/EB below

- In the Susztak lab, tubule-specific CD36 expression will be studied, in an attempt to generate more tubulointerstitial injury and fibrosis. In addition based on the new results endothelial specific CD36 transgenic animals will also be generated

- The finding that defects in oxphos are associated with podocyte loss is quite interesting, and the identification of genes within the particular genetic regions that have been identified is an important goal. With regard to systemic anti-oxidant treatment, the selection of agent and dose may be critical, as well as a way to monitor oxidant status in plasma or tissue – otherwise success or failure in ameliorating disease may be hard to interpret. The experiments to reduce by half complex I transcripts is high risk, as the mice may have a phenotype prior to induction of diabetes; nevertheless, if successful in converting the B6 mouse to nephropathy prone, this will be an important observation. With regard to the Susztak work, the hypothesis that CD36 contributes to tubulointerstitial fibrosis is interesting and the transgenic mice should answer the question.

R13/EB: As described in the Annual Report, we have identified endothelial cells as the likely source cell type for OXPHOS inhibition and mitochondrial dysfunction with oxidative DNA damage. These results very clearly rule out podoyctes as the target cell, and suggest that in response to bioenergetics crisis and oxidative damage endothelial cells may generate

permissive or instructive proapoptotic and/or detachment signals to promote podoycte depletion. Because of these findings, we revised our approach and did not pursue experiments to reduce Complex I level using podocyte-specific conditional gene deletion.

- Below is a list of your AMDCC publications from the website. Should any publications be added or subtracted? Has all of the relevant data from these publications been uploaded to the website? Please work with Dr. Rick McIndoe to ensure that the website and database are up-to-date and complete.

The following publications should be added:

Chua SC, Li Y, Liu SM, Liu R, Chan KT, Martino J, Susztak K, Zheng Z, D'Agati V, Gharavi AG: Mapping a Susceptibility Gene for Diabetic nephropathy to Mouse Chromosome 8 in db/db FvB/NJ, a Robust Mouse Model of Diabetic Kidney Disease Kidney Int. 2010 Sep;78(5):453-62

Ahn SA and Susztak K: Understanding Diabetic Kidney Disease is getting a Notch closer Diabetes, 2010 Aug;59(8):1865-7.2010

Chang GY, Susztak K: Closing up on glomerular insulin signaling in diabetic kidney disease Kidney International *2011 Apr;79(8):802-4*

Human and murine kidneys show gender- and species-specific gene expression differences in response to injury.
Si H, Banga RS, Kapitsinou P, Ramaiah M, Lawrence J, Kambhampati G, Gruenwald A, Bottinger E, Glicklich D, Tellis V, Greenstein S, Thomas DB, Pullman J, Fazzari M, Susztak K.
PLoS One. 2009;4(3):e4802. Epub 2009 Mar 11.

Laser capture microdissection of kidney tissue.
Woroniecki RP, Bottinger EP.
Methods Mol Biol. 2009;466:73-82.

Decorin deficiency enhances progressive nephropathy in diabetic mice.
Williams KJ, Qiu G, Usui HK, Dunn SR, McCue P, Bottinger E, Iozzo RV, Sharma K.
Am J Pathol. 2007 Nov;171(5):1441-50. Epub 2007 Sep 20.

Molecular profiling of diabetic mouse kidney reveals novel genes linked to glomerular disease.
Susztak K, Böttinger E, Novetsky A, Liang D, Zhu Y, Ciccone E, Wu D, Dunn S, McCue P, Sharma K.
Diabetes. 2004 Mar;53(3):784-94.

Genomic strategies for diabetic nephropathy.
Susztak K, Sharma K, Schiffer M, McCue P, Ciccone E, Böttinger EP.
J Am Soc Nephrol. 2003 Aug;14(8 Suppl 3):S271-8. Review.

The following publication should be subtracted:

1. Lights on for aminopeptidases in cystic kidney disease.
   Bottinger EP
   The Journal of clinical investigation, 2010 (120(3)), 660 - 663