AMDCC Annual Report (2011)

PI: BROSIUS, FRANK
Project Title: Recaptulating transcriptional pathways of human DN in mice
Grant Number: U01 DK076139

Abstract: Valid murine models of diabetic nephropathy (DN) should replicate the molecular changes and not simply the pathological alterations of patients with DN. Thus, our general hypothesis for development and testing of murine models of DN is that Current murine models fail to show human-like DN because they fail to replicate glomerular and tubulointerstital gene expression changes that occur in humans with progressive DN. Replication of the critical transcriptomic profiles of patients with progressive DN should induce progressive DN in mice. Our use of data in human DN generated by the European Renal cDNA Bank (ERCB) will be critical in testing and validating the mouse models of the Animal Models of Diabetic Complications Consortium. We have performed initial transcriptomic analyses of humans with DN using the ERCB to identify pathways which are reliably altered in humans but not in murine models. One pathway that is consistently altered in glomeruli and tubulointerstium in diabetes in humans, but not in mice, is the JAK/STAT pathway. Expression of all JAK members was increased when confirmed with real time PCR analysis. We have focused on JAK2 given its key role in mediating responses implicated in DN. Moreover, JAK2 is activated by reactive oxygen species and interacts with PPAR(signaling, both of which are implicated in DN. For our 2 novel models, we propose podocyte and proximal tubular-specific Jak2 transgenic db/m C57BLKS mice. For these and other models in the Consortium we propose to: 1. Determine whether transcriptional changes in humans are reproduced in the glomerular and tubulointersitial compartments of the Jak2//db/db BLKS models, and other AMDCC models; 2. Determine if all the pathologic and pathophysiologic features of human DN are replicated in the Jak2//db/db BLKS models; 3. Determine if JAK2/3 inhibitors prevent development of DN in the Jak2 transgenic models and other good candidate models that replicate human transcriptomic changes; 4. Determine if ROS production drives JAK2 expression in glomerular and/or tubulointerstitial compartments and enhances downstream JAK2 signaling and whether JAK2 expression promotes ROS; 5. Determine if PPAR(agonists prevent J.ak2 downstream effects in glomerular and/or tubulointerstitial compartments. This research is directly relevant to the study and prevention of diabetic kidney disease, the major cause of kidney failure in the U.S. By creating and understanding a mouse model that develops human-like diabetic kidney disease, we can then move rapidly to tests of strategies to prevent and cure this disease.

1. Program Accomplishments:

Hypothesis: ***Current murine models fail to show human-like DN because they fail to replicate glomerular and tubulointerstitial gene expression changes that occur in humans with progressive DN. Replication of the critical transcriptomic profiles of patients with progressive DN should induce progressive DN in mice.***

Progress toward stated aims:

1. Generation and analysis of podocyte specific JAK2 transgenic mice. In order to generate the most reliable model of enhanced JAK2 expression and one that would be of most use to other investigators, we opted for an approach in which a STOP/FLOX JAK2 construct has been "knocked-in" to the ROSA26 locus. We used the pBigT/pROSA26PA targeting system from the Costantini lab (Columbia University)(1) and inserted the sequences for *JAK2* in the targeting system. Once recombined into the ROSA26 locus, this allowed generation of mice with cell-type specific overexpression of the JAK2 transgene by crossing the mice with tissue specific Cre mice. Expression of Cre recombinase excises the "floxed" stop codon in front of the *JAK2* sequence and permits *JAK2* transcription driven by the ubiquitously expressed ROSA26 promoter. Because of the moderately enhanced sensitivity of the 129S6/SvEvTac strain (compared to C57BL/6) to DN, we bred our targeted mutation onto this background and established the NPHS2 (podocin) Cre mouse on the same background. With the researchers at The Jackson Laboratory we have confirmed that both transgenic models are on a pure 129S6/SvEvTac background. These mice are now available to the AMDCC from JAX. We have produced Ins2 $^{Akita/+}$ JAK2$^{loxP/loxP}$ mice which have been crossed to the NPHS2 Cre mice to produce triple heterozygote Ins2 $^{Akita/+}$ JAK2$^{loxP/+}$ NPHS2Cre/+ as well as double heterozygote JAK2$^{loxP/+}$ NPHS2Cre/+ mice for use in our experiments to test the effects of podocyte specific JAK2 overexpression in type 1 diabetes. These JAK2 heterozygote mice have a ~80% increase in total glomerular JAK2 mRNA expression which is similar to the increase seen in humans with progressive DN (2). The relative increase of JAK2 expression in podocytes necessarily must be somewhat higher but is not easily quantifiable. With this relatively modest increase in JAK2 expression, there was a significant increase in albuminuria in diabetic podocyte JAK2 mice at 12 wks of age compared to diabetic control littermates (Fig. 1). There was no difference in albuminuria between podocyte JAK2 and control nondiabetic mice. However, the difference in albuminuria disappeared by 24 weeks and was also not present at 32 weeks. Mesangial expansion was also not greater in the podocyte JAK2

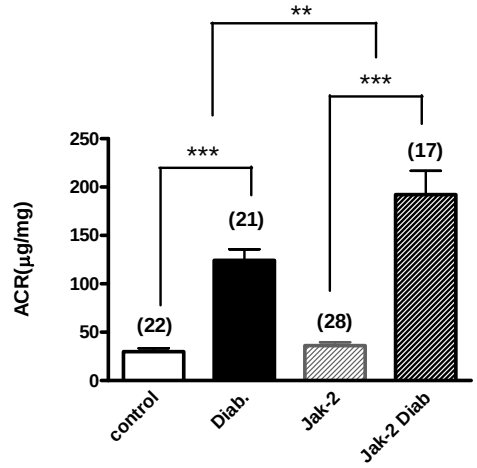

Fig. 1. Albuminuria in control and Akita (Diab) wild-type and podocyte JAK2 (Jak-2) mice showing a 66% greater increase in albuminuria at 12 wks in the Akita JAK2 compared to the Akita wild-type mice (**p < 0.01).

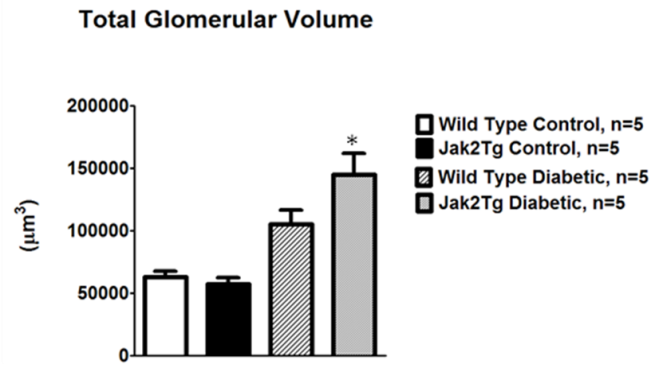

Fig. 2. Total Glomerular Volume in control and Akita (diabetic) wild-type and podocyte JAK2 (Jak2Tg) mice showing a greater glomerular volume in the Akita JAK2 compared to the Akita wild-type mice (Bonferroni correction ,*p < 0.05 vs. wild-type diabetic).

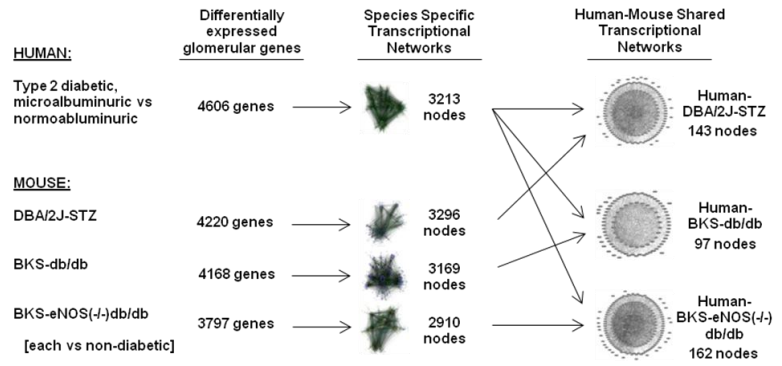

Fig. 2. DEGs from glomeruli of human and 3 mouse models of DN were identified (left), arranged into transcriptional networks (mid) and then the human-mouse profiles were examined for shared networks (right). Multiple shared networks were identified.

diabetic mice than in the wild-type diabetic mice at 32 weeks of age. However, there was a substantial increase in glomerular volume in the diabetic JAK2 mice compared with the wild-type diabetics. This suggests that podocyte JAK2 may be more involved in glomerular growth than in fibrosis.

2. <u>Transcriptomic analysis of glomeruli from humans and 3 mouse models of DN</u>. To facilitate the transcriptomic cross-species comparison of DN in humans and mice, four species-specific transcriptional networks were generated from differentially expressed glomerular genes from the human group and the three mouse models versus the respective controls (Fig. 2). The transcriptional networks integrate phenotyping and gene expression with known and predicted transcriptional regulation as well as prior knowledge using the Genomatix Bibliosphere algorithm (http://www.genomatix.de/download/software/Bibliosphere_manual.pdf) . Genes were represented as nodes connected by edges that represent node-node dependencies. Using the graphical matching tool, TALE (Tool for Approximate LargE graph matching) (3), the human glomerular transcriptional network was overlaid on each mouse glomerular transcriptional network to define similar structured subnetworks, thus generating three shared human-mouse transcriptional networks. At each step of the analysis, the number of genes and nodes decreased, demonstrating that the analysis reduced network complexity while iteratively identifying species-specific and cross-species differentially regulated genes (Fig. 2).

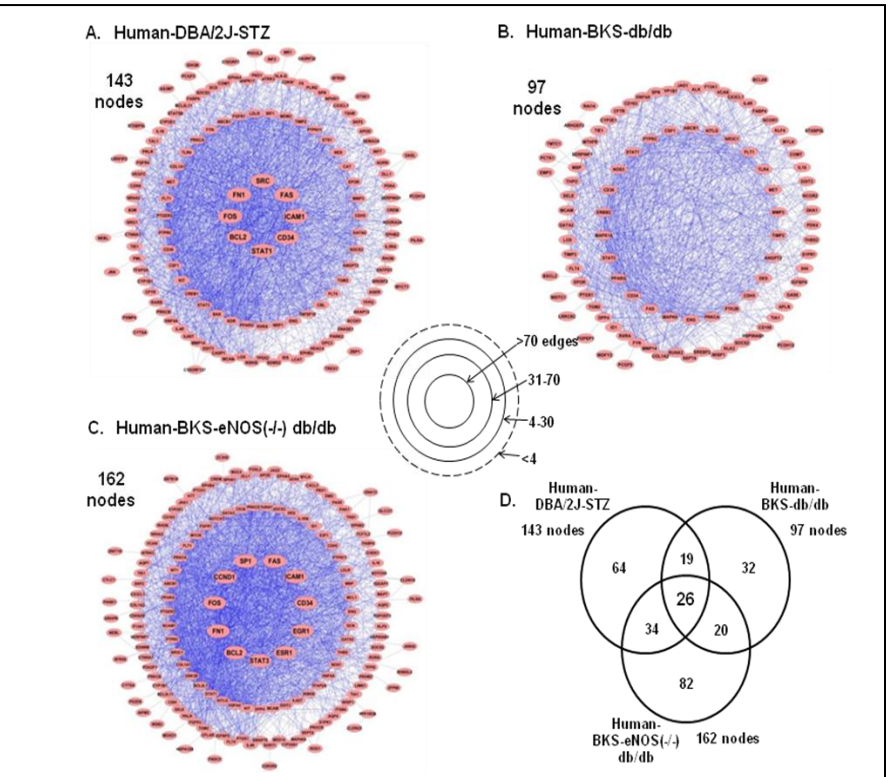

Fig. 3. Human-mouse shared transcriptional DN networks showing the shared nodes (genes) and edges (functional connections between genes) in the 3 comparisons (panels A-C). The most higly connected nodes are near the center as shown by the concentric circle key in the center of the figure. Overlap between the 3 comparsions is shown in panel D.

Figure 3 graphically displays the human-mouse shared transcriptional DN networks. Each shared network was arranged in concentric circles based upon the number of edges (connections) for each node, with the most highly connected nodes nearest the center. More node-node connections suggest increased network importance or centrality. The number of nodes in the Human-DBA STZ (Figure 3A) and the Human-BKS db/db eNOS(-/-) transcriptional networks (Figure 3C) were 143 and 162 respectively, each with high connectivity nodes (> 70 edges), and overlapped one another in 60 nodes (Figure 3D). The Human-BKS db/db transcriptional network

contained fewer nodes, no nodes with > 70 edges, and overlapped with the other two networks in 45 and 46 nodes. A total of 26 nodes were shared by all three human-mouse networks (Figure 3D).

Some high connectivity nodes were shared in all 3 networks. For example, STAT1 and STAT3, members of the JAK/STAT pathway, were among the top nodes in all three shared networks. In addition, genes expressed by endothelial cells and associated with endothelial cell dysfunction, including CD34, CD36, and FLT1, were high connectivity genes in each shared network. Other gene nodes in the top 30 list were present in only two networks. Shared networks were analyzed by the Bibliosphere algorithm to examine original transcriptional network relationships.

To maximize detection of shared pathways and cooperating genes, the human-mouse glomerular transcriptional networks in Fig. 3 display overlapping differentially regulated genes without accounting for the direction of differential expression in cross-species comparison. In each shared network, the

Table 1: Top canonical pathways in shared human-mouse DN network analysis

Canonical pathway	# Total genes	Human-DBA/2J-STZ # Genes observed	Human-BKS-db/db # Genes observed	Human-BKS-eNOS (-/-) db/db # Genes observed
Cytokine receptor degradation signaling (INOH:JAK_STAT_pathway_and_regulation)	272	27	17	21
AKT(PKB)-Bad signaling (INOH:EPO_signaling)	178	18	12	13
IL-7 signaling pathway (JAK1 JAK3 STAT5) (INOH:IL-7_signaling)	177	15	9	11
Migration (INOH:VEGF)	180	15	9	11
Signaling events mediated by VEGFR1 and VEGFR2 (NCI-nature:vegfr1_2_pathway)	66	11	8	8
FGF signaling pathway (NCI-nature:fgf_pathway)	63	9	4	6
HIF-1-alpha transcription factor network (NCI-nature:hif1_tfpathway)	68	8	4	7
Angiopoietin receptor Tie2-mediated signaling (NCI-nature: angiopoietinreceptor_pathway)	49	6	5	---
Signaling events activated by Hepatocyte Growth Factor Receptor (c-Met) (NCI-nature:met_pathway)	55	6	4	---
Regulation of nuclear SMAD2/3 signaling (NCI-nature:smad2_3nuclearpathway)	83	---	5	9
Regulation of Androgen receptor activity (NCI-nature:ar_tf_pathway)	52	---	6	5
IL6-mediated signaling events (NCI-nature:il6_7pathway)	45	8	---	8
IL-2 receptor beta chain in t cell activation (BioCarta:il2rbpathway)	49	6	---	7
EGFR1 (CellMap:EGFR1)	157	10	---	---
Signaling events mediated by PTP1B (NCI-nature:ptp1bpathway)	52	7	---	---
Alpha6Beta4Integrin (CellMap:Alpha6Beta4Integrin)	49	---	5	---
Endothelins (NCI-nature:endothelinpathway)	61	---	5	---
C-MYB transcription factor network (NCI-nature:cmyb_pathway)	88	---	---	9
Androgen Receptor (CellMap:AndrogenReceptor)	87	---	---	8

number of concordantly expressed genes (i.e., when the change in expression of a human gene was in the same direction as that of the orthologous mouse gene) is lower than the number of discordantly expressed genes. The percentages of concordantly expressed genes were as follows: in the Human-DBA STZ transcriptional network, 28% (40 out of 143); in the Human-BKS db/db transcriptional network, 45% (44 out of 97) and in the Human-BKS db/db eNOS(-/-),31% (50 out of 162). This relative lack of concordance suggests different adaptations to initial gene expression changes between mouse and humans, and could potentially explain some of the differences between the progressive DN in humans and lack of such progression in mice, but this interpretations is speculative.

Pathway enrichment analysis was performed for the gene node list from each human-mouse comparison to assess the biologic relevance of the cross-species network. All three shared networks were highly enriched for canonical JAK-STAT signaling, which agrees with and extends our previous AMDCC findings (2). Canonical pathways well described in DN, such as VEGF receptor and FGF signaling, and HIF-1 gene regulation pathways are also shared among all three networks. In contrast, the IL7 signaling pathway has not been investigated in DN to our knowledge. Table 1 lists the top canonical pathways including those enriched in one or two transcriptional networks, such as the c-myb transcriptional factor network enriched in Human-BKS db/db eNOS(-/-) comparison. Thus, this approach can be useful in screening several mouse models at once to identify the best model to investigate specific molecular targets relevant to human DN. A total of 26 genes were shared by all three networks. Pathway enrichment analysis with these genes yielded a similar list of enriched canonical pathways, such as canonical JAK-STAT signaling, VEGF receptor signaling, the HIF-1 gene regulation pathway as did the TALE-based approach. However, the pathway enrichment analysis approach was less informative than the TALE-based approach as fewer genes from each canonical pathway were identified, and some canonical pathways were not identified (e.g., the IL-7 signaling pathway).

To highlight canonical pathways most relevant to DN progression, the degree of change in ACR and GFR for each human subject was correlated with the relative expression of each gene found in the enriched pathways. An increase in ACR correlated with enhanced expression of 6 genes and with decreased expression of 12 genes while a decline in GFR correlated with an increased expression of 3 genes and a decline in expression of 2 genes. Moreover, the JAK-STAT signaling pathway and the HIF-1 gene regulation pathway contained the highest number of genes of all the canonical pathways for which expression correlated with disease progression.

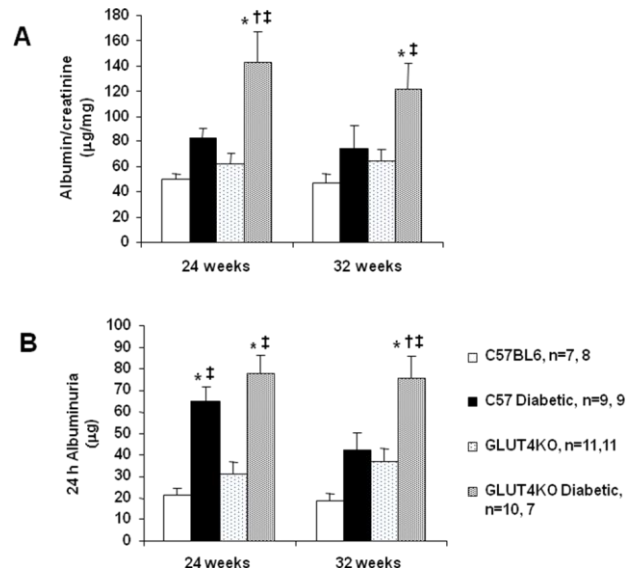

Fig. 4. Albuminuria in diabetic and control total body GLUT4KO and wild-type (C57BL6) mice after 24 and 32 weeks of diabetes. ($p<0.05$ vs. *C57BL/6 nondiabetic, † C57BL/6 diabetic, ‡GLUT4KO nondiabetic mice).

A manuscript reporting these findings will be submitted in the next month.

3. Final analysis of GLUT4 -/- and podocyte specific GLUT4 -/- mice. These models were developed many years ago as part of the initial AMDCC project and have now been completely analyzed. At the initiation of these experiments none of us recognized how resistant the C57BL/6 mice are to nephropathy and in retrospect it would have helped to have these mice on a more nephropathic background. We did not have resources to do this, however, and the studies were mostly completed in any case. We found that total body knock out of GLUT4 (tbGLUT4KO) resulted in lower fasting blood

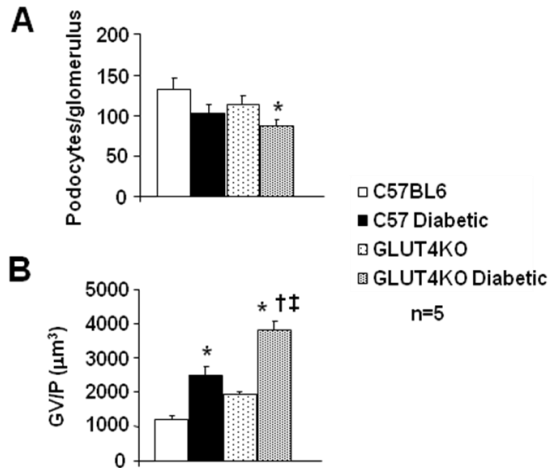

Fig. 5. Podocyte number per glomerulus (A) and glomerular volume per podocyte (inverse of density) (B) in diabetic and control total body GLUT4KO and wild-type (C57BL6) mice after 32 weeks of diabetes ($p<0.05$ vs. *C57BL/6 nondiabetic, † C57BL/6 diabetic, ‡GLUT4KO nondiabetic mice).

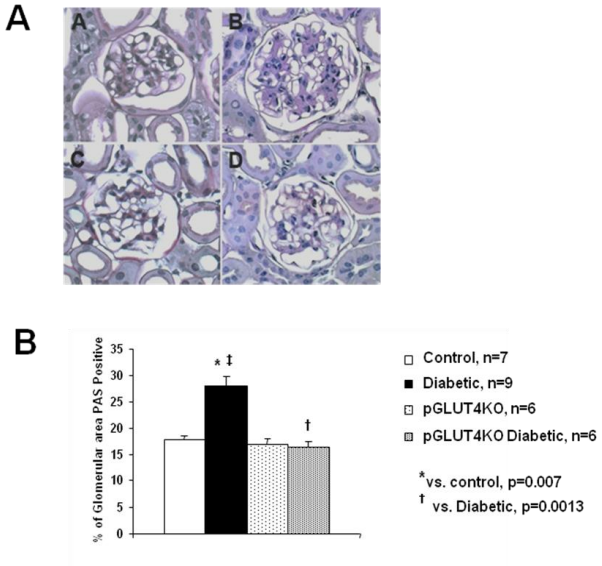

Fig. 6. Mesangial matrix (expressed as percent of glomerular area that was PAS positive) in control (A, C) and diabetic (B, D) wild-type and podocyte specific GLUT4 knockout (pGLUT4KO) mice.

glucose and glycosylated hemoglobin values than those of the GLUT4 wild-type diabetic animals. The tbGLUT4KO diabetic mice also had a significantly lower urine volume than wild-type diabetic mice at 32 weeks, commensurate with their lower blood sugars and, presumably, lower glycosuria. The tbGLUT4KO diabetic mice, despite consistently lower blood glucose levels, showed significantly more albuminuria than C57BL/6 diabetic mice (Fig. 4A). Twenty four hour urinary albumin excretion paralleled the uACR data (Fig. 4B). Mesangial matrix expansion also paralleled these findings but the differences between the tbGLUT4KO mice and wild-type diabetic mice were not significant. The tbGLUT4KO diabetic mice showed a significant decrease in podocyte number compared to C57BL/6 control mice, again along with a significant increase in glomerular volume. The GV/P (glomerular volume per podocyte), an inverse of density that depicts the amount of glomerular volume covered by each podocyte, was significantly higher in the tbGLUT4KO mice compared to all other groups (Fig. 5). Podocyte number appeared to be inversely correlated with albuminuria in tbGLUT4KO animals, but this correlation was not statistically significant ($r^2=0.316$, $p=0.1151$).

In the podocyte specific GLUT4 knockout (pGLUT4KO) experiments, there were no statistically significant differences in albuminuria in any group at 24 or 32 weeks of diabetes, though the wild type diabetic animals tended to have increased albuminuria and the pGLUT4KO diabetic mice had values similar to that of nondiabetic mice. Similarly, the mesangial matrix was significantly expanded in wild-type diabetic mice compared to controls but the mesangial matrix areas were essentially identical in the pGLUT4KO diabetic mice and non-diabetic controls (Fig. 6). The mean number of podocytes in glomeruli of diabetic, pGLUT4KO and pGLUT4KO diabetic mice was significantly less than that in control nondiabetic mice. Total glomerular volume was significantly increased in the control diabetic group only. Podocyte density was significantly reduced in diabetic and pGLUT4KO diabetic groups from all controls. pGLUT4KO diabetic mice had a significantly lower podocyte density than the control diabetic animals (Fig. 7). Podocyte number was inversely correlated with albuminuria in pGLUT4KO animals (pGLUT4KO diabetic and nondiabetic mice: $r^2=0.402$, $p=0.0491$). Although the slope of this relationship was similar to that of total body GLUT4KO animals, the line was displaced inferiorly,

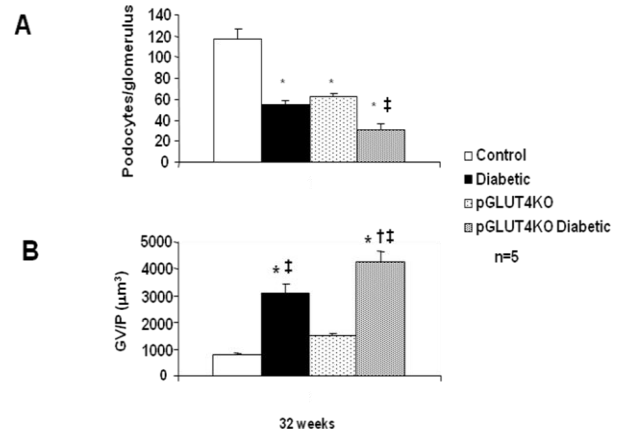

Fig. 7. Podocyte number per glomerulus (A) and glomerular volume per podocyte (inverse of density) (B) in diabetic and control pGLUT4KO and wild-type mice after 32 weeks of diabetes ($p<0.05$ vs. control nondiabetic, † C57BL/6 diabetic, ‡GLUT4KO nondiabetic mice).

suggesting that fewer podocytes were present for any comparable degree of albuminuria in pGLUT4KO animals.

This manuscript will be submitted in the next 2 months pending decisions about collaboration and co-submission with a similar manuscript with Dr. Alessia Fornoni (University of Miami).

Plans for the year and completion of the project:

Our major focus in the few months remaining in the grant will be to finalize characterization of the heterozygote podocyte JAK2 transgenic mouse and perform initial characterization of the homozygote JAK2 mice. We will also treat diabetic wild-type and JAK2 mice with Ang II to see if activation of JAK-STAT signaling by a pathophysiologically relevant factor will augment DN in the podocyte JAK2 mice. We will also make arrangements for deposit of AMDCC model embryos at JAX or other long-term storage facilities.

2. <u>Collaborations:</u>

With other AMDCC PIs: Drs. Brosius and Kretzler continue to work in a highly interactive manner with the laboratory of Dr. Eva Feldman. Drs. Kretzler and Brosius worked closely with Dr. Ray Harris on the AMDCC project on the transcriptomic analysis of the eNOS -/- db/db mouse glomeruli, reported above, and nerve samples. Drs. Thomas Coffman and Susan Gurley were critical in the generation of the 129S6/SvEvTac mouse lines for the JAK2 STOP/FLOX mice and appropriate Cre mice. The project on the podocyte GLUT4 knockout mice continues work with Dr. Dale Abel. Dr. Susztak was very helpful in helping work out issues with the PEPCK Cre mouse.

Our AMDCC studies helped launch another collaborative effort to utilize mouse models for systems biological approaches to diabetic complications. Drs. Kretzler, Feldman and myself, along with Dr. Hosagrahar Jagadish, in Electrical Engineering and Computer Science, and Dr. Sub Pennathur, in Internal Medicine, have joined forces (as multiple principal investigators) in a NIDDK sponsored R24 grant entitled, "Integrated Systems Biology Approach to Diabetic Microvascular Complications." This grant was funded and has led to important new transcriptomic and metabolomic analyses re: validation of pathways involved in progressive diabetic complications.

With Jax: The final characterization of the pure background of the JAK2 STOP/FLOX mice was performed at JAX as was the final breeding and validation of the podocin Cre mice onto the 129S6/SvEvTac background. Finally, JAX bred the Akita mutation into stocks to allow for generation of the necessary triple heterozygote mice (Cre/+ JAK2/+ Akita/+) mice for our experiments.

With the MMPCs: none

With other non-AMDCC PIs: See above for the R24 grant collaborations. We work also closely with Dr. Christin Carter-Su (University of Michigan) and members of her laboratory on JAK/STAT signaling aspects, and with Dr. Sub Pennathur on oxidative markers, metabolomics and proteomics in diabetic complications (University of Michigan). Dr. Brosius has continued close collaboration with Dr. Charles Heilig (University of Florida) on GLUT1 overexpression models of diabetic nephropathy which resulted in 2 publications in Am J Physiol in 2010. Drs. Brosius and Dr. Fornoni (University of Miami) are sharing data on our mutual podocyte GLUT4 knockout mice and will try to publish results

together. Drs. Brosius and Kretzler continue collaborations on DN with numerous investigators internationally.

3. <u>Address previous EAC comments:</u>

A. *Effect of podocyte specific GLUT1 overexpression. An earlier AMDCC mouse model was fully characterized within the last year. Has this data been uploaded to the website?*

Yes. Dr. Heilig has also agreed to upload the total body GLUT1 overexpression data.

B. *How will transcriptomic data (from the P&F) be presented and shared via the website?*

We cannot upload Pima Indian data due to IRB constraints. Animal data will be uploaded.

C. *The gene expression network comparisons between mice and humans are interesting. The finding that Glut1 over-expression leads to diabetic renal lesions in non-diabetic mice, but protects against them in diabetic mice is strange and needs more discussion. Completion of the phenotyping of the JAK2 Tg mice is needed, but the initial findings do not seem encouraging. The productivity is good.*

The disparate findings re: total body vs podocyte-specific GLUT1 overexpression are probably due to the site of expression. In the total body GLUT1 overexpressers, the promoter was chosen to induce expression primarily in mesangial cells. Thus, the likely reason for the differences in these 2 models is that the "total body" GLUT1 animal phenotype is largely due to mesangial cell GLUT1 overexpression and the podocyte-specific model clearly reflects effects of GLUT1 on the podocyte. These cell type specific responses are interesting, but not especially surprising. The GLUT4 knockout models show similar divergence (see above) and also suggest an inverse effect of GLUT1 and GLUT4 on each cell type, suggesting differential effects of each transporter on cell behavior.

D. *The website is set-up to display microarray data. Has all of the data from your P&F (with Dr. Kretzler) and primary award been uploaded to the site?*

See above.

E. Ongoing progress is reasonable. Difficulties in sufficiently upregulating Jak2 expression in target compartments. Continued phenotyping of these animals and continuing analysis of transcriptomic comparisons between mouse and human for upcoming year.

We agree with the concern about modest JAK2 overexpression. We are augmenting by breeding homozygous STOP/FLOX mice to test for DN. We have continued our transcriptomic analyses as noted above and these are ready for submission.

F. Two projects are on-going. First the generation and characterization of JAK2 transgenic mice, expressed in proximal tubule (disappointingly low transgene expression) and podocyte (produced at JAX, now being crossed with Akita). Second, transcriptional studies are underway comparing glomeruli from db/db, db/db ENOS, and streptozotocin mice. The preliminary data, presented here in a fragmentary form, support the role of JAK/STAT in the db/db ENOS model. Overall, progress has been satisfactory, with the most promising data coming from the "omics" approaches. Beyond acidification of the drinking water, another approach to increasing PEPCK promoter activity is a high protein diet. The omic studies are a major strength, particularly given the rich database of human kidney biopsy materials available to Dr. Kretzler. Expansion of the omics studies, made possible by the recently funded R24, will likely complement the current project with proteomic and metabolomic studies.

Thank you for these comments with which we agree, and for the suggestion re: stimulation of the PEPCK promoter. Given the difficulties noted by us and the EAC, we have decided to concentrate on the podocyte specific model, where we have a phenotype, albeit a muted one at best.

I have not presented the data from the R24 project which have indeed pointed to new metabolic pathway alterations in complications-prone tissues in early diabetes in humans and in diabetic murine models, and have suggested that levels.

4. Publications

1. Brosius FC. Susceptible mice: identifying a diabetic nephropathy disease locus using a murine model. Kidney Int. 2010;78:431-2.
2. Duan SZ, Usher MG, Foley EL 4th, Milstone DS, Brosius FC 3rd, Mortensen RM.Sex dimorphic actions of rosiglitazone in generalised peroxisome proliferator-activated receptor-gamma (PPAR-gamma)-deficient mice. Diabetologia. 2010 Jul;53(7):1493-505.
3. Ju W, Brosius FC 3rd. Understanding kidney disease: toward the integration of regulatory networks across species. Semin Nephrol. 2010;30(5):512-9
4. Kumar PA, Brosius FC 3rd, Menon RK. The Glomerular Podocyte as a Target of Growth Hormone Action: Implications for the Pathogenesis of Diabetic Nephropathy. Curr Diabetes Rev. 2010 Nov 10. [Epub ahead of print]
5. Abnormalities in signaling pathways in DN.
   Brosius FC, Khoury CC, Buller CL, Chen S
   Expert review of endocrinology & metabolism, 2010 (5(1)), 51 – 64
6. Transgenic Overexpression of GLUT1 in Mouse Glomeruli Produces Renal Disease Resembling Diabetic Glomerulosclerosis. Wang Y, Heilig KO, Saunders T, Minto AW, Deb DK, Chang A, Brosius FC 3rd, Monteiro C, Heilig CW. *Am J Physiol Renal Physiol, 2010 (PMID: 20375117)*
7. Podocyte Specific Overexpression of GLUT1 Surprisingly Reduces Mesangial Matrix Expansion in DN in Mice. Zhang H, Schin M, Saha J, Burke K, Holzman LB, Filipiak WE, Saunders T, Heilig CW, Brosius FC 3rd. *Am J Physiol Renal Physiol, 2010 (PMID: 20375116)*
8. A New Pair of SOCS for DN. Brosius FC 3rd, Banes-Berceli A. *J Am Soc Nephrol. 2010 May ;21(5):723-4 (PMID: 20413610)*
9. The management of diabetic neuropathy in CKD.
   Pop-Busui R, Roberts L, Pennathur S, Kretzler M, Brosius FC, Feldman EL
   American journal of kidney diseases : 2010 (55(2)), 365 – 385
10. Mouse Models of DN:A Midstream Analysis from the Animal Models of Diabetic Complications Consortium
    Frank C. Brosius IIIa, Charles E. Alpersb, Erwin P. Bottingerc, Matthew D. Breyerd, ThomasM. Coffmane, Susan B. Gurleye, Raymond C. Harrisf, Masao Kakokig, Matthias Kretzler, Edward H. Leiterh, Moshe Levii, Richard A. McIndoej, Kumar Sharmak, Oliver Smithiesg, Katalin Susztakl, Nobuyuki Takahashig, Takamune Takahashif
    Journal of the American Society of Nephrology : JASN, 2009 (20(12)), 2503 - 2512
11. Enhanced Expression of JAK-STAT Pathway Members in Human DN
    Celine C. Berthier, PhD., Hongyu Zhang, M.D., MaryLee Schin, B.S., Anna Henger, PhD, Robert G. Nelson, M.D., PhD, Berne Yee, M.D., Anissa Boucherot, PhD., Christin Carter-Su, PhD., Lawrence S. Argetsinger, PhD., Maria Pia Rastaldi, M.D., Frank C. Brosius, M.D., Matthias Kretzler, M.D
    Diabetes, 2008 (Epub), 469 - 477
12. New insights into the mechanisms of fibrosis and sclerosis in DN.
    Brosius FC. *Reviews in endocrine & metabolic disorders*, 2008 (9(4)), 245 - 254
13. Rosiglitazone reduces renal and plasma markers of oxidative injury and reverses urinary metabolite abnormalities in the amelioration of DN
    Hongyu Zhang, Jharna Saha, MaryLee Schin, Jaeman Byun, Matthias Kretzler, Eva L. Feldman, David A. Weild, Subramaniam Pennathur, Frank C. Brosius III
    American journal of physiology. Renal physiology, 2008 (295(4)), F1071 - F1081

14. Rosiglitazone Treatment Reduces Diabetic Neuropathy in STZ treated DBA/2J mice
    Timothy D Wiggin, Matthias Kretzler, Subramaniam Pennathur, Kelli A. Sullivan, Frank C Brosius, Eva L Feldman
    Endocrinology, 2008 (149(10)), 4928 - 4937
15. From fibrosis to sclerosis: mechanisms of glomerulosclerosis in DN.
    Qian Y, Feldman E, Pennathur S, Kretzler M, Brosius FC
    Diabetes, 2008 (57(6)), 1439 - 1445
16. Mouse models of diabetic neuropathy.
    Sullivan KA, Hayes JM, Wiggin TD, Backus C, Su Oh S, Lentz SI, Brosius F, Feldman EL
    Neurobiology of disease, 2007 (28(3)), 276 - 285
17. Reduction in Podocyte Density as a Pathologic Feature in Early DN in Rodents: Prevention of by Lipoic Acid Treatment
    Brian Siu, Jharna Saha, William E. Smoyer, Kelli A. Sullivan, Frank C. Brosius III
18. Role for GLUT1 in diabetic glomerulosclerosis.
    Heilig CW, Brosius FC, Cunningham C
    Expert reviews in molecular medicine, 2006 (8(4)), 1
19. DN: of mice and men.
    Breyer MD, Böttinger E, Brosius FC, Coffman TM, Fogo A, Harris RC, Heilig CW, Sharma K
    Advances in chronic kidney disease, 2005 (12(2)), 128 - 145
20. Glucose transporters in DN.
    Brosius FC, Heilig CW
    Pediatric nephrology (Berlin, Germany), 2005 (20(4)), 447 - 451
21. Mouse Models of DN
    MATTHEW D. BREYER, ERWIN BÖTTINGER, FRANK C. BROSIUS, III, THOMAS M. COFFMAN, RAYMOND C. HARRIS, CHARLES W. HEILIG, AND KUMAR SHARMA (FOR THE AMDCC)
    Journal of the American Society of Nephrology : JASN, 2005 (16), 27 – 45

5. References

1. Srinivas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F: Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1:4, 2001
2. Berthier CC, Zhang H, Schin M, Henger A, Nelson RG, Yee B, Boucherot A, Neusser MA, Cohen CD, Carter-Su C, Argetsinger LS, Rastaldi MP, Brosius FC, Kretzler M: Enhanced expression of Janus kinase-signal transducer and activator of transcription pathway members in human diabetic nephropathy. *Diabetes* 58:469-477, 2009
3. Tian Y, Patel JM: A Tool for Approximate Large Graph Matching. *The 24th International Conference on Data Engineering (ICDE)*, 2008