Animal Models of Diabetic Complications Consortium
(U01 DK076134)

Annual Report
(2009)

Novel mouse models of diabetic nephropathy: Role of FXR
University of Colorado Denver

Principal Investigator
Moshe Levi

Address: 12700 East 19th Avenue, Research 2, Aurora, Colorado 80045
Phone: 303-724-4825
E-mail: Moshe.Levi@ucdenver.edu

Table of Contents

Animal Models of Diabetic Complications Consortium
(U01 DK076134)

Part A:

Principal Investigator's Summary

1. Program Accomplishments:

Hypothesis:

The hypothesis of our proposal was that FXR deficiency especially in nephropathy susceptible genetic backgrounds will result in accelerated diabetic nephropathy.

Progress toward stated milestones:

We obtained the FXR generalized knockout mice on the C57BL/6 genetic background we submitted them to JAX from them to a) backcross into FVB, DBA-2J and SvEv129 genetic backgrounds, and b) cross breed with the Akita mice with type 1 diabetes and db-db mice with type 2 diabetes.

In the interim in our lab we generated a FXR knockout mouse colony on the nephropathy resistant C57Bl/6 genetic background and we fed them a western diet (high fat and cholesterol) and we induced hyperglycemia with streptozotocin, 50 mg/kg, IP, 5 consecutive doses.

We found that at the end of the 2 month period FXR KO diabetic mice have a 10-fold increase in urinary albumin to creatinine ratio (Figure 1), they have hypertrophic glomeruli and develop renal tubular lesions (Figure 2), they have increased macrophage infiltration in the glomeruli (Figure 3), they have altered expression of podocyte staining (Figure 4), they have a relative increase in GBM thickness and podocyte foot process effacement (Figure 5), they have increased accumulation of fibronectin and increased staining of alpha-smooth cell actin (Figure 6), increased Mason Trichrome staining suggestive of tubulointerstitial fibrosis (Figure 7), increased expression of TGF-beta 1, alpha-smooth cell actin, and fibroblast specific protein-1 (Figure 8), increased microRNA 192 and decreased microRNA 29a (Figure 9).

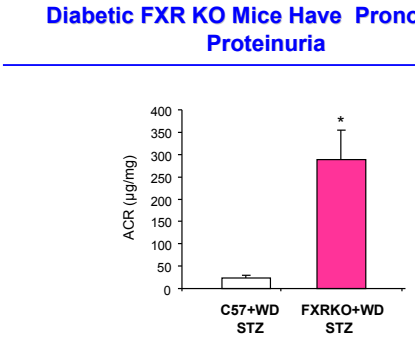


Figure 1

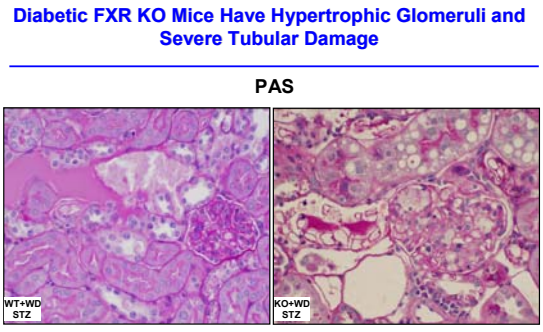


Figure 2

Diabetic FXR KO Mice Have Significantly More Macrophage Infiltration in Glomeruli

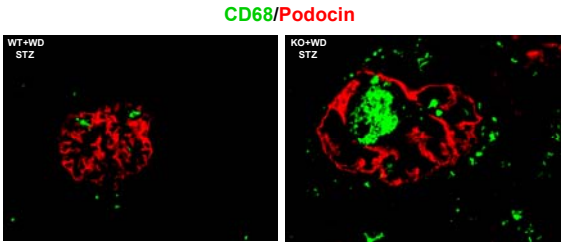


Figure 3

Diabetic FXR KO Mice Lose Normal Podocyte Density

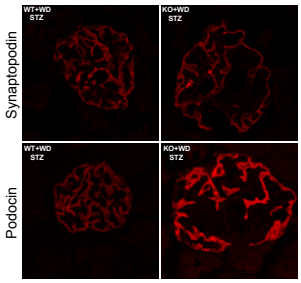


Figure 4

Diabetic FXR KO Mice Have Thickened GBM and Foot Process Effacement

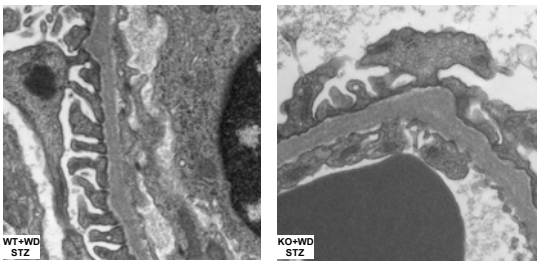


Figure 5

Diabetic FXR KO Mice Have Increased Expression of Matrix Protein and Fibrosis Markers

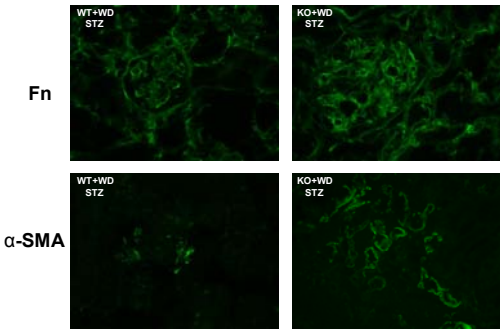


Figure 6

Diabetic FXR KO Mice Have Increased Tubulointerstitial Fibrosis

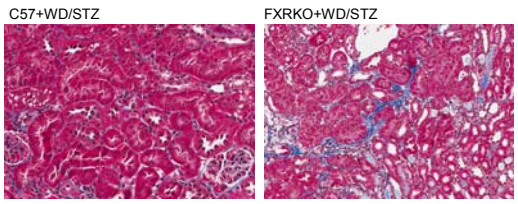


Figure 7

Diabetic FXR KO Mice Have Increased Expression of Profibrotic Growth Factor and Fibrosis Markers

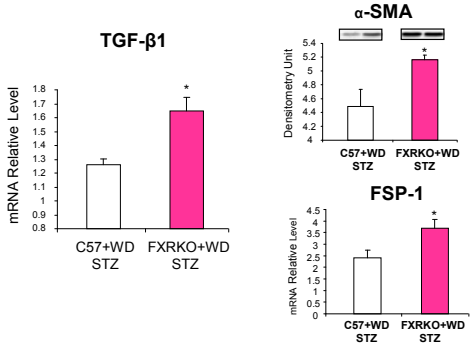


Figure 8

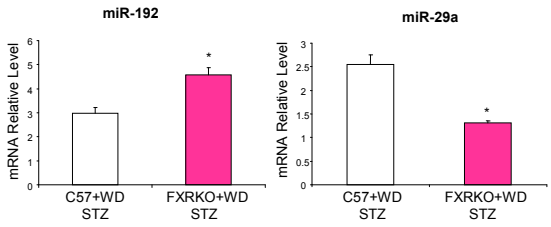


Figure 9

In addition in the FXR KO diabetic mice we found increased oil red o staining in the glomeruli and tubular cells (Figure 10), which correspond to increases in renal triglyceride and cholesterol content (Figure 11). These lipid alterations are mediated by increased SREBP-1c mediated fatty acid and triglyceride synthesis (Figure 12) and increased cholesterol uptake mediated by LDLR and LOX-1 (Figure 13).

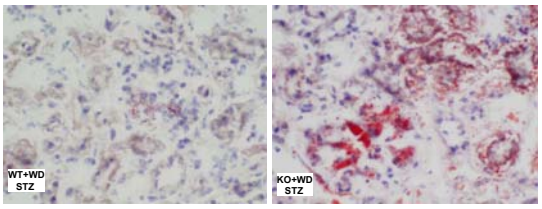


Figure 10

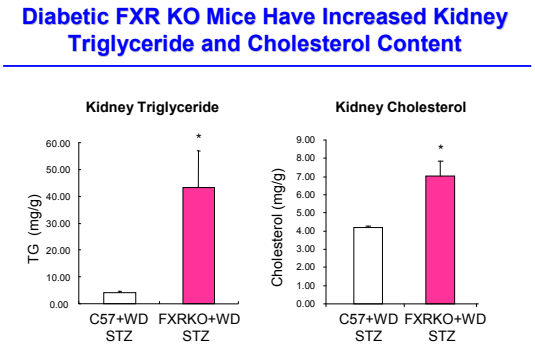


Figure 11

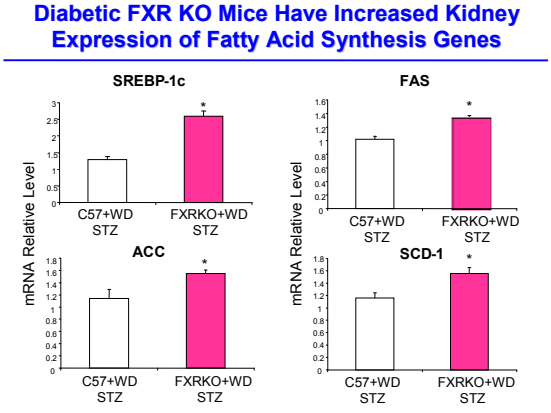


Figure 12

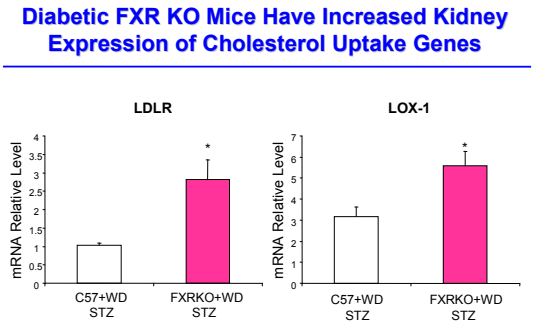
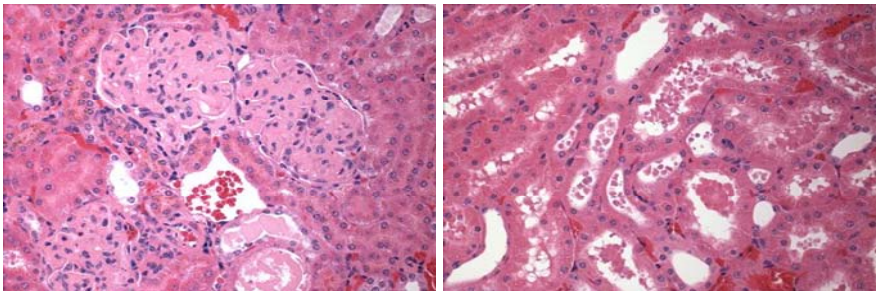


Figure 13

The increase in SREBP-1c in the FXR KO diabetic mice is quite significant as in prior studies we have determined that increased expression of SREBP-1 per se (SREBP-1 transgenic mice) mediates lipid accumulation as well as inflammation, oxidative stress, and fibrosis in non-hyperglycemic and non-hyperlipidemic mice. In contrast the renal effects of diet induced obesity and diabetes is significantly attenuated in SREBP-1 KO mice.

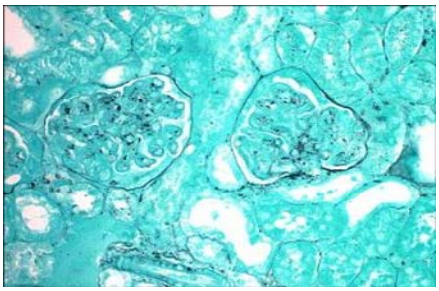
Jackson Labs has recently generated the FXR KO mice in the FVB genetic background and they have started to cross them with the Akita mice. Preliminary studies indicate that the female FXR KO mice on FVB genetic background develop prominent glomerular lesions yet to be defined and the male FXR KO mice on the FVB genetic background also develop glomerular lesions and have marked lipid accumulation in the glomeruli.

Female 09-520: FXR-/-, wild type at *Ins2* (e.g., not Akita) Nephropathy

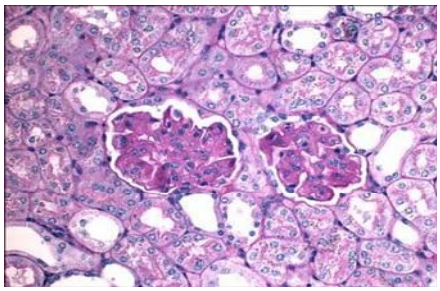


H&E staining shows global glomerular hyalinization and tubular changes

Female 09-520: FXR-/-, wild type at *Ins2* (e.g., not Akita) Nephropathy

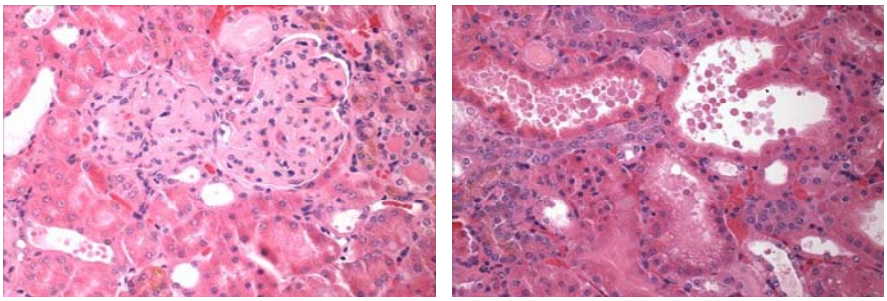


Silver methenamine stain shows no unusual GBM thickening



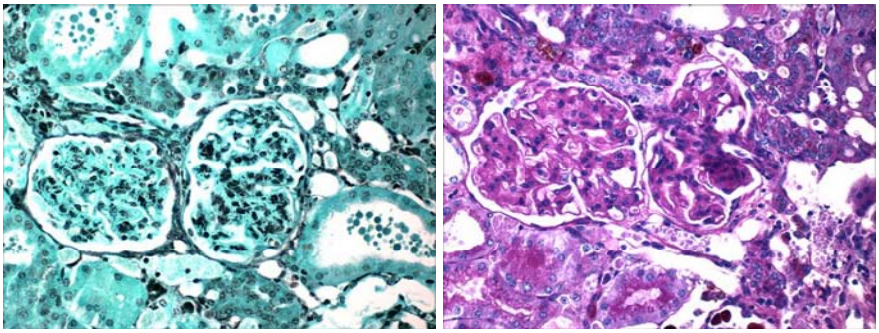
PAS stain shows increased PAS positive material in the glomerular tuft, but no unusual GBM thickening

Female 09-521: FXR-/-, *Ins2*^{Akita}/+



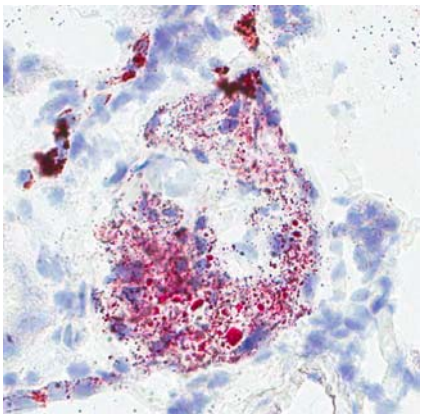
Glomeruli and tubules show the same pathologic changes as in 09-520

Female 09-521: FXR-/-, *Ins2*^{Akita}/+



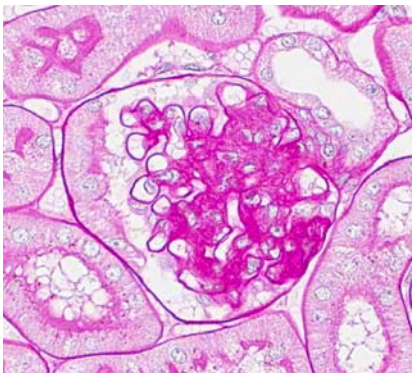
Silver methenamine and PAS stains show the same features as in 09-520

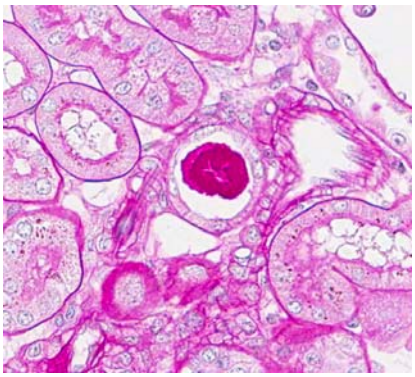
Male 09-688: FXR-/-, *Ins2*^{Akita}/+



Oil red O stains show the fat droplets

Male 09-688: FXR-/-, *Ins2*^{Akita}/+





PAS stain shows increased PAS positive material in the glomerular tuft and hyalinization

Plans for the Upcoming Year:

The above studies indicate that while FXR KO mice have a 10-fold increase in proteinuria they do not develop robust classical glomerular lesions of diabetic glomerulosclerosis. We are in discussions to determine alternate mechanisms for the genesis of glomerular proteinuria as well as possibly determining the potential role of the proximal tubule in the resultant albuminuria.

The above studies were conducted in 6 month old FXR KO mice. To rule out age-dependent changes we will also conduct studies in 2 month old FXR knockout mice and in these studies we will also measure blood pressure by telemetry as FXR has also been shown to have vascular effects.

In addition we will continue to characterize the FXR KO mice on the FVB genetic background generated by JAX and the FXR KO mice crossed with Akita mice.

2. Collaboration:

With other AMDCC PIs:

We are in the process of examining the pathways which the other AMDCC PIs have determined to play an important role in the pathogenesis of diabetic nephropathy. Of great significance we have found that FXR modulates eNOS and Bradykinin 2 Receptor which Ray Harris and his group at Vanderbilt and Oliver Smithies and his group at University of North Carolina at Chapel Hill has determined to be critical for diabetic nephropathy.

In this regard in collaboration with Ray Harris we are starting to study the effects of FXR activating ligands in db-db mice, eNOS KO mice and db-db x eNOS double KO mice. These studies were inspired by our finding that FXR modulated eNOS in the kidney.

In addition in collaboration with Oliver Smithies we will study the effects of FXR activating ligands in Akita mice, Bradykinin B1 and B2 Receptor KO mice and Akita x B1B2 KO mice. These studies were inspired by our finding that FXR modulated B2 Receptor in the kidney.

Since we have also been successful in generating FXR transgenic mice future studies can also employ these mice to determine the effects of FXR per se in the diabetic models.

With JAX:

JAX is generating the FXR KO mice on the FVB, DBA-2J and SvEv129 genetic backgrounds and they are crossing them onto the Akita and db-db mice on the parallel genetic backgrounds.

With the MMPCs:

We are working with the Seattle MMPC for the kidney pathology studies. In the future we will also consider working with the metabolic phenotyping MMPCs to fully determine carbohydrate and lipid metabolism in the FXR KO mice.

With other non-AMDCC PIs:

During the past academic year Richard Johnson moved from U of Florida to U of Colorado and we will start using our mice to determine for the presence of additional pathways which they have determined to play an important role in diabetic nephropathy.

3. Address previous EAC comments:

COMMENTS: The Levi lab studies the role of the nuclear hormone receptor, FXR, in diabetic complication pathogenesis. Given the role of abnormal fatty acid metabolism in the pathogenesis of diabetic complications, phenotyping FXR null and over-expressing diabetic mice (goals of this project) should provide interesting results. Progress has been adequate in generating the mouse strains needed for these studies. Several aims will completed by end of 2008 and the remaining consortium funding will be used to phenotype the "FXR-manipulated" animals after crossing with type 1 and type 2 DM models.

REPLY: These studies are in progress.

COMMENTS: The FXR KO seems to have a strong phenotype and interaction with diet. Is there any effect on arterial pressure?

REPLY: In the current studies we will also carefully monitor arterial blood pressure.

COMMENTS: The plans to do more microscopy seem warranted.

REPLY: In addition to light microscopy we are also performing immunofluorescence microscopy and electron microscopy analyses of the kidney samples.

4. Publications:

1. Villa-Bellosta R, Barac-Nieto M, Breusegem SY, Barry NP, **Levi M**, and Sorribas V: Protein interactions of the growth-related, type IIc renal Na/phosphate co-transporter: Kidney International 73: 456–464, 2008. NIHMSID 122098

2. Faroqui S, **Levi M**, Soleimani M, and Amlal H: Estrogen downregulates the expression of proximal tubule Na-Pi cotransporter (NaPi-IIa) and causes phosphaturia in ovariectomized rats: Kidney International 73:1141-50, 2008 NIHMSID 122152

3. **Levi M**: Novel NaPi-2c mutations that cause mistargeting of NaPi-2c protein and uncoupling of Na-Pi cotransport cause HHRH. American J Physiology Renal Physiology 295: F369-F370, 2008 PMC Journal in Process.

4. **Levi M** Breusegem SY: Renal Phosphate–Transporter Regulatory Proteins and Nephrolithiasis. New England Journal of Medicine 359: 1171-1173, 2008. NIHMSID 122153

5. Kratzer A, Buchebner M, Pfeifer T, Becker TM, Uray G, Miyazaki M,Miyazaki-Anzai S, Ebner S, Chandak PG, Kadam RS, Calayir E, Rathke N, Ahammer H, Radovic B, Trauner M, Hoefler G, Kompella UB, Fauler G, **Levi M**, Levak-Frank S,Kostner GM, and KratkyG: Synthetic LXR agonist attenuates plaque formation in ApoE-deficient mice without inducing liver steatosis and hypertriglyceridemia J. Lipid Res. 50: 312-326, 2009. PMC2636920

6. Villa-Bellosta R, Ravera S, Sorribas V, Stange G, **Levi M**, Murer H, Biber J, Forster IC. The Na+-Pi cotransporter PiT-2 (SLC20A2) is expressed in the apical membrane of rat renal proximal tubules and regulated by dietary Pi. American J Physiology Renal Physiol. 296:F691-9, 2009 PMC2670642

7. Breusegem SY, Takahashi H, Giral-Arnal H, Wang X, Jiang T, Verlander JW, Wilson P, Miyazaki S, Sutherland E, Caldas Y, Blaine JT, Segawa H, Miyamoto K, Barry NP, and **Levi M**. Differential Regulation of the Renal Sodium/Phosphate Co-Transporters NaPi-IIa, NaPi-IIc and PiT-2 in Dietary Potassium Deficiency. Am J Physiol Renal Physiol. 297:F350-61, 2009

8. Villa-Bellosta R, **Levi M**, and Sorribas V: Vascular smooth muscle cell calcification and SLC20 inorganic phosphate transporters: effects of PDGF, TNF- and Pi. Pflugers Archiv-European Journal of Physiology: 2009 Jun 9. [Epub ahead of print] PMID: 19506901

9. Giral-Arnal H, Caldas Y, Sutherland E, Wilson P, Breusegem SY, Barry NP, Blaine JT, Jiang T, Wang XX, **Levi M**: Regulation of the Rat Intestinal Na-dependent Phosphate Transporters by Dietary Phosphate: Am J Physiol Renal Physiol. 2009 Aug 12. [Epub ahead of print] PMID: 19675183

10.Brosius FC, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, Harris RC, Kakoki M, Kretzler M, Leiter EH, **Levi M**, McIndoe RA, Sharma K, Smithies O, Susztak K, Takahashi N, Takahashi T, and AMDCC: Mouse Models of Diabetic Nephropathy: JASN 2009.

11. Blaine JT, Okamura K, Giral-Arnal H, Breusegem SY, Caldas Y, Barry NP, **Levi M**: PTH Induced Internalization of Apical Membrane NaPi-2a: Role of Action and Myosin VI: Am J Physiol Cell Physiol.

12. Wang XX, Jiang T, Shen Y, Adorini L, Pruzanski M, Gonzalez FJ, Lewis L, Miyazaki-Anzai S and **Levi M**: Farnesoid X Receptor Modulates Renal Lipid Metabolism and Diet-Induced Renal Inflammation, Fibrosis and Proteinuria: Am J Physiol Renal Physiol.