Mouse Models of Diabetic Nephropathy

Mount Sinai / Jefferson / Einstein / Minnesota

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Group Members

- *Mount Sinai: Phenotyping, Molecular Pathology & Validation*
  - Erwin Bottinger (Team Leader)
  - Katalin Susztak, Co-Investigator
  - Che-Kang Li, Technician
- *Albert Einstein: Strain Generation & Characterization*
  - Maureen Charron (Team Leader)
  - Scott Henderson, Technician
- *Thomas Jefferson: Experimental Diabetes, HPLC Core, Phenotyping*
  - Kumar Sharma (Team Leader)
  - Steve Dunn, Technician and Core Manager
  - Steven Williams,
  - Peter McCue, Pathology
- *U Minnesota: Phenotyping (Morphometry)*
  - Michael Steffes, Consultant

Work-In-Progress

- *Strain Generation*
  - Tie1-GLUT1 tg: ↑ endothelial glucose transport / glucotoxicity
  - Nphs2-GLUT1 tg (FVB): ↑ podocyte glucose transport / glucotoxicity
  - gGT-CD36 tg: ↑ AGE&FFA induced apoptosis in tubules

- *Experimental Models*
  - db/db C57BLKS longitudinal, standardized phenotyping
  - Decorin knockout with low dose STZ
  - flk-1 RAGEtg with low dose STZ
  - Cd2ap+/- with Akita

db/db C57BLKS/J
AMDCC Phenotyping & Model Validation

- To validate features of human diabetic nephropathy in db/db C57BLKS using AMDCC Procedures & Standards
- Study the progression and molecular fingerprints of kidney disease in the db/db mice
- Mice analyzed 4,8,12,20,28 weeks of age

	4 weeks	8 weeks	12 weeks	20 weeks
Body weight (gr)				
$Lepr^{db/m}$	19.1±0.9	27.8±2.4	31.1±3.3	35.2±3.8
$Lepr^{db/db}$	25.8±1.6**	41±5.4**	46.9±1.6**	45.4±4.1**
Kidney weight (mg)				
$Lepr^{db/m}$	149.5±13	167±16	195±23	196±27
$Lepr^{db/db}$	166±17	184±34*	226±24*	255±29**
Blood Glucose (mg/dl)				
$Lepr^{db/m}$	149±17	130±30	169±27	128±33
$Lepr^{db/db}$	133±78	530±83**	541±74**	565±63**
Albuminuria (ug/24 hr)				
$Lepr^{db/m}$	39.9±26	35.5±15	67±30	51±18
$Lepr^{db/db}$	82±38**	43±22*	162±58**	151±81**
Creatinine (serum) (ug/dl)				
$Lepr^{db/m}$	32±23	59±47	91±23	102±3
$Lepr^{db/db}$	52±23	37±14	84±33	35±13**
GFR (ul/min/mouse)				
$Lepr^{db/m}$	150±119	177±83	198±72	129±121
$Lepr^{db/db}$	189±66	157±102	107±100	370±259*

	4 weeks	8 weeks	12 weeks	20 weeks
Mesangial Matrix Score (AU)				
$Lepr^{db/m}$	1.09±0.13	1.33±0.23	1.17±0.12	1.17±0.06
$Lepr^{db/db}$	1.27±0.16	1.63±0.4	1.81±0.32*	3.2±0.5**
GBM thickness (nm)				
$Lepr^{db/m}$	131±14	134±3	144±1	150±10
$Lepr^{db/db}$	135±8	146±7	162±17	173±7*
Glomerular volume (um^3)				
$Lepr^{db/m}$	133±39	139±37	159±29	199±33
$Lepr^{db/db}$	150±42	206±86	245±42*	261±35*
Endocapillary apoptosis/glomerular cross section				
$Lepr^{db/m}$	0±0	0.41±0.34	0.69±0.43	0±0
$Lepr^{db/db}$	0±0	1.56±1.21	0.54±0.49	0.91±0.78
Podocyte apoptosis/glomerular cross section				
$Lepr^{db/m}$	0.32±0.23	0.98±0.69	0.94±0.37	0.80±0.66
$Lepr^{db/db}$	0.21±0.16	5.56±1.7**	3.92±1.66**	2.75±1.39*
Podocyte number/glomerular cross section				
$Lepr^{db/m}$	12.2±1.3	11.5±2.4	10.9±0.7	11.6±0.45
$Lepr^{db/db}$	12.8±0.28	9.8±0.8	8±0.27**	9.2±0.89*

Glomerular Molecular Fingerprints in Diabetic Kidneys

Purity- and Quality-Controlled Glomerular Preps (3 mice pooled)
AFFYMETRIX 430 Mouse Whole Genome Arrays
Standardized Data Analysis Algorithms

Differentially expressed genes 1% FDR 1.5x change in both MAS5 and RMA

lepr$^{db/db}$ vs. lepr$^{db/m}$	372
Diabetic vs. control	587
Albuminuria vs. control	273
Mesangial expansion vs. control	1290

Work ongoing with Rick McIndoe's Group to add data to AMDCC Website

Db/db Conclusions

- ***AMDCC Nephropathy Model Validation Criteria***
  - **Present: Glomerulopathy**
    - Podocyte Apoptosis (8 wks)
    - Albuminuria (12 wks)
    - Podocyte Depletion (12 wks)
    - Mesangial Expansion (12 wks)
    - Glomerular Volume (12 wks)
    - GBM Thickening (20 wks)
  - **Absent: Tubulointerstitial and Vascular Lesions**
    - No Tubulointerstitial disease
    - No Nodular lesions
    - No Arteriolar hyalinosis
  - To Be Resolved: Renal Function
- ***New Insights Relevant to Human DNP***
    - Podocyte apoptosis peak coincides with onset of hyperglycemia
    - Podocyte depletion precedes/coincides with urinary albumin excretion

High Glucose Causes Apoptosis in Cultured Murine Podocytes

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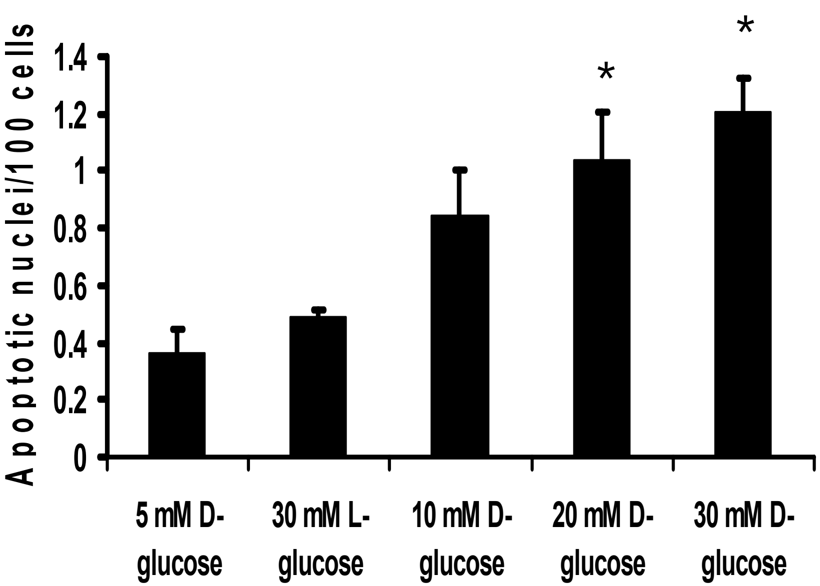
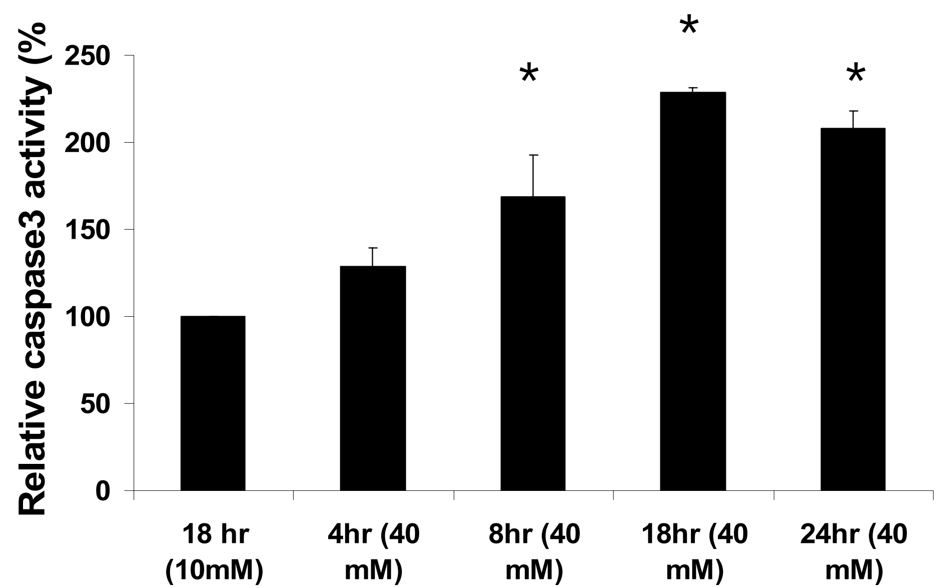
Podocyte Apoptosis: Dose Response



Podocyte Apoptosis: Time Course



NOVEL DATA I: GLOMERULAR

Podocyte Apoptosis:
An Early Lesion in Diabetic Nephropathy

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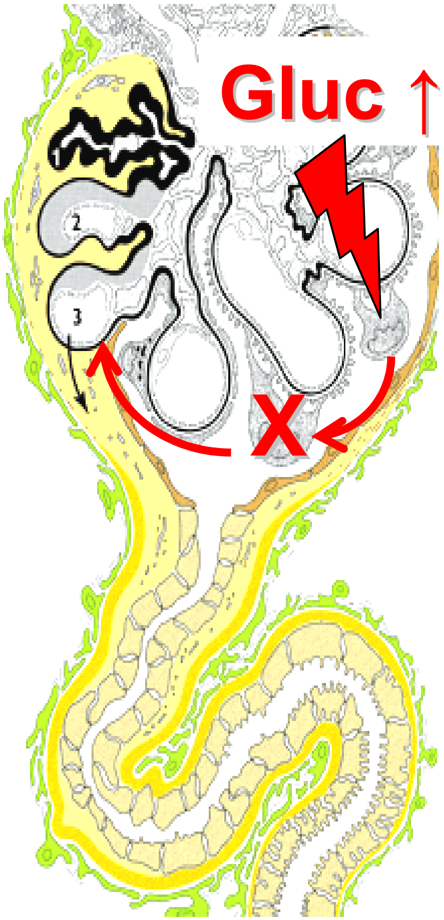


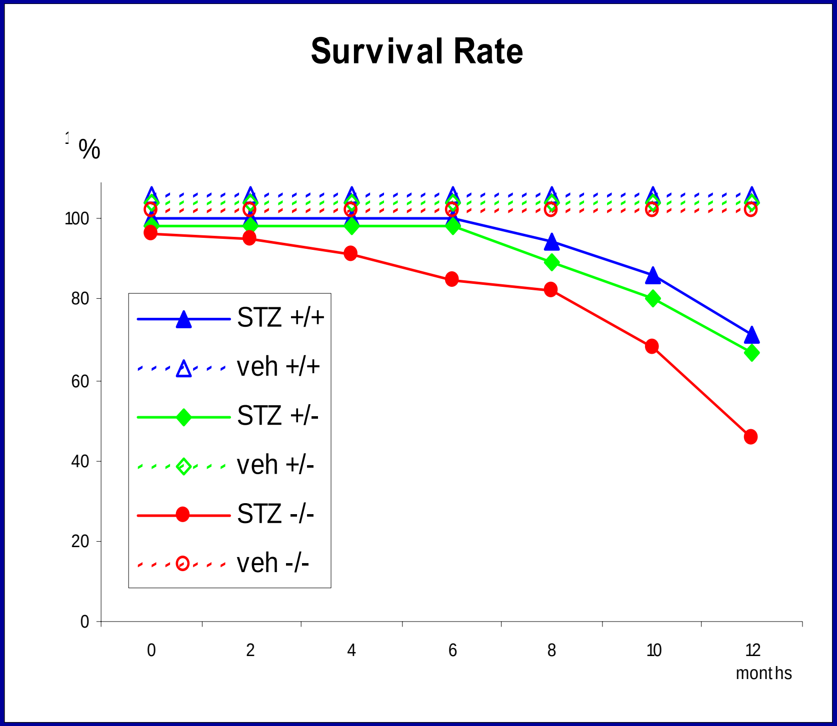
- **Glucose** is sufficient to cause apoptosis in cultured podocytes
- **Podocyte apoptosis**
  - coincides with onset of hyperglycemia in T2D mice
  - causes loss of podocytes (depletion)
  - In T2D mouse, podocyte depletion is not progressive

Decorin Knockout and Diabetic Complications

Decorin and Diabetic Nephropathy

- Background: Decorin is a small proteoglycan that inhibits TGF-β via direct binding to active TGF-β. Decorin is stimulated in human mesangial cells by high glucose and stimulated in several mouse models of diabetic kidney disease.
- Hypothesis: Decorin protects against diabetic kidney disease.
- Back-cross *Dcn* KO allele onto the C57BL/6J background; induce STZ diabetes in male littermates with different *Dcn* gene doses
  - Albuminuria
  - Plasma creatinine by HPLC
  - Histologic parameters of diabetic nephropathy
  - EM analysis of GBM and TBM

Decorin KO Diabetic Mice Have Excess Mortality

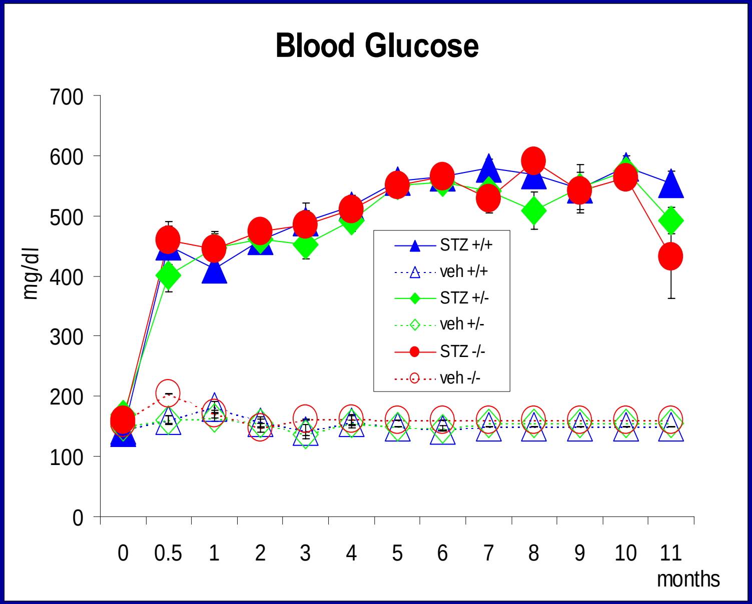


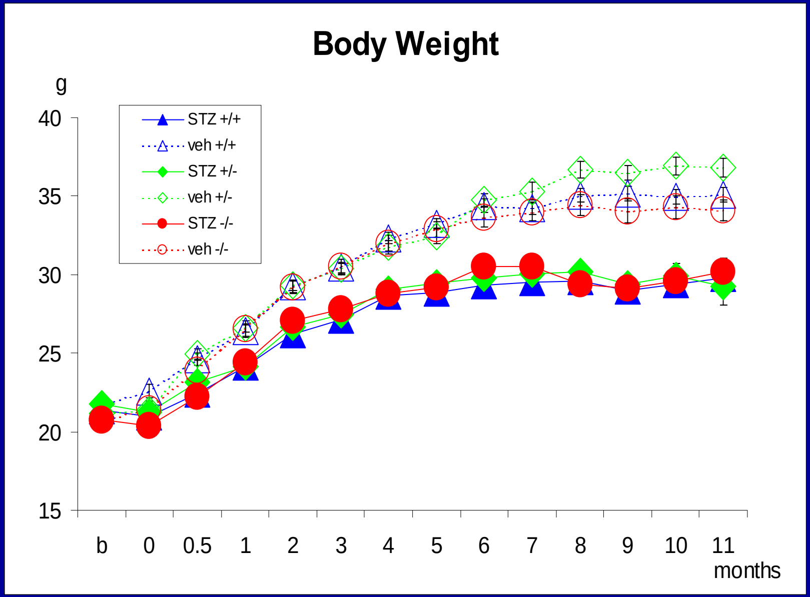
- Over ½ (12/22) of decorin KO diabetic mice died spontaneously by 12 months of diabetes
- Wild type diabetic mice had less mortality by 12 months of diabetes (4/14)
- The diabetic mice that died were characterized by increased plasma creatinine and increased albuminuria compared to mice that survived up to 12 months

Clinical Parameters of Diabetic Mice that Died Spontaneously

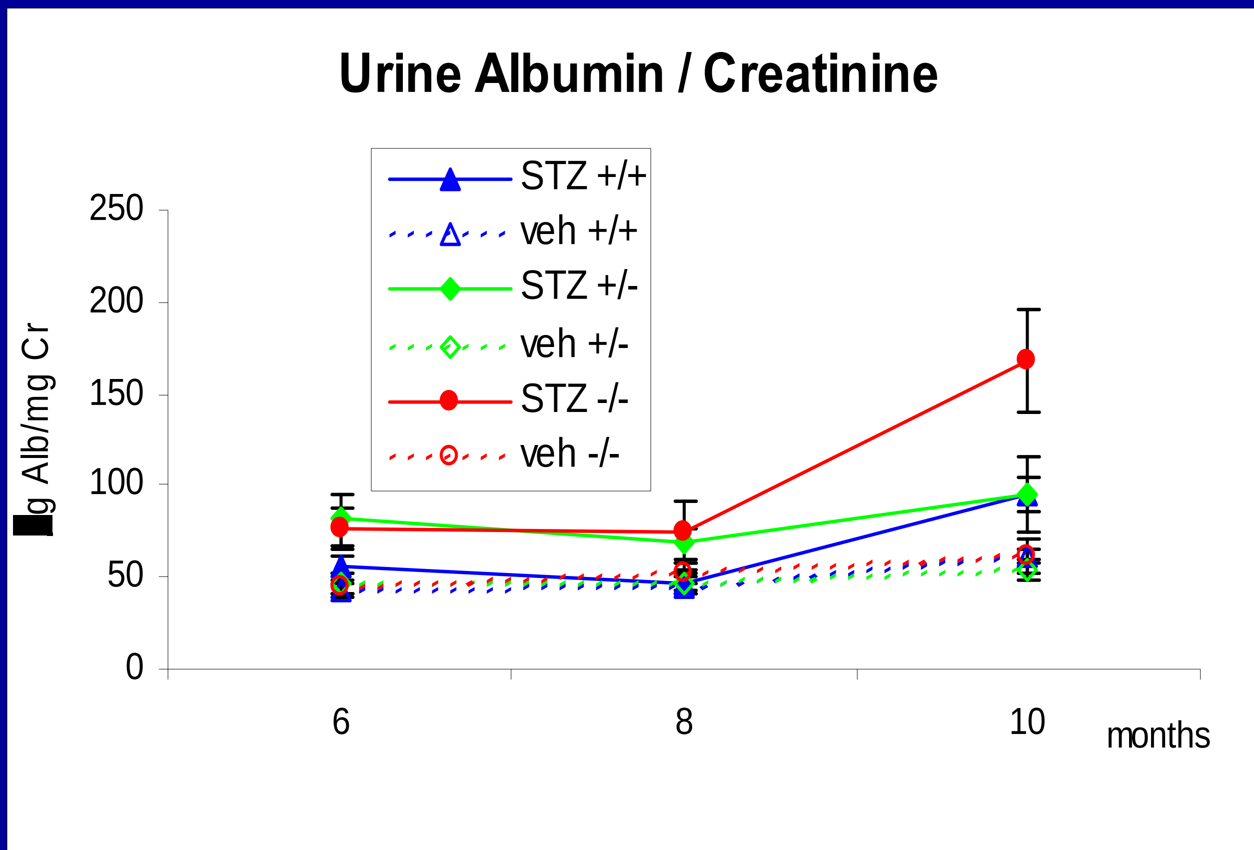
	Diabetic Mice (survived, n=30)	Diabetic Mice (died <12 m, n=21)
Body wt (g)	29.17 ± 0.65	29.96 ± 0.57
Blood gluc (mg/dl)	499 ±32.3	553.85 ±17.0
STZ dose	333 ±24.6	314 ±24.7
Urine alb/creat	98.8 ±11.7	115.52 ±21
Plasma Creat (mg/dl)	0.22 ±0.01	0.27 ±0.02

Blood Glucose and Body Weight in Diabetic Mice

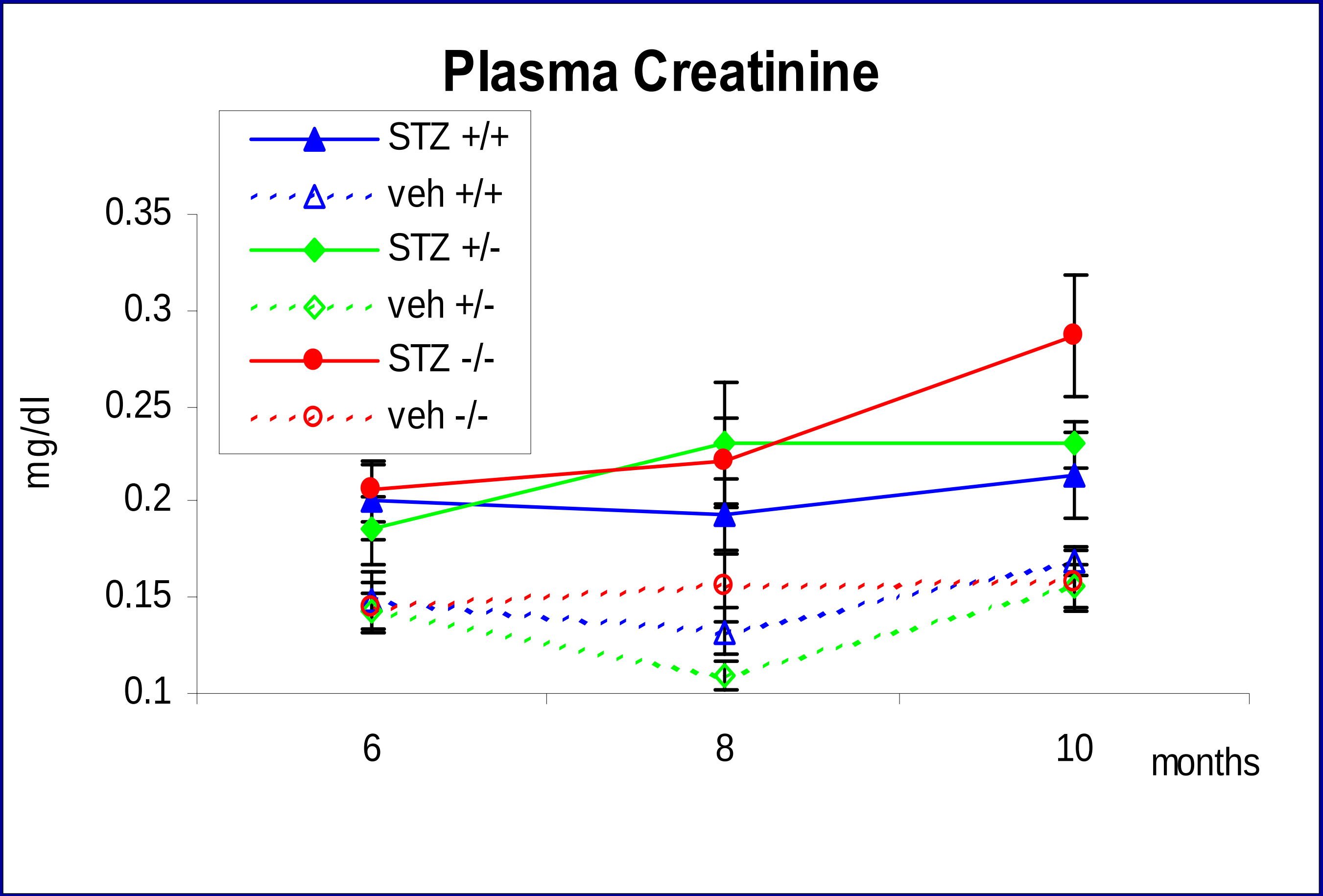




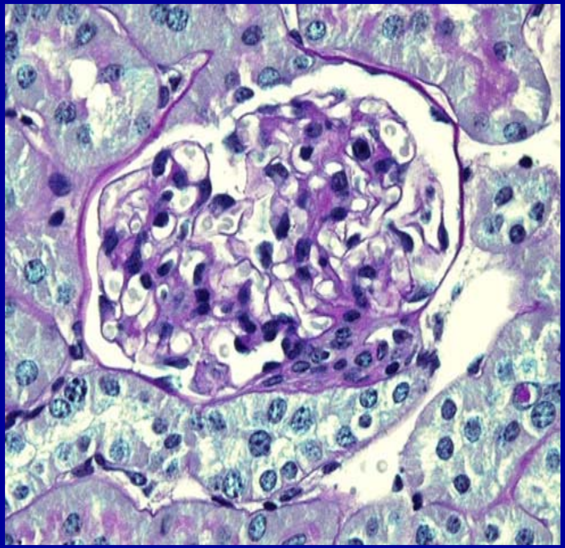
Loss of Decorin Results in Increased Diabetic Albuminuria



Loss of Decorin Contributes to Renal Dysfunction in Diabetes

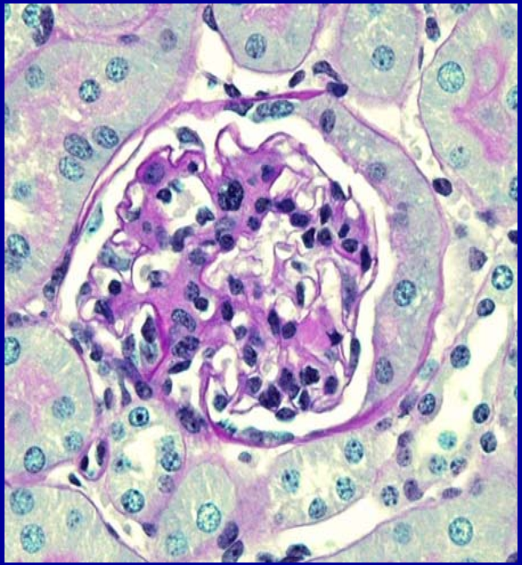


Loss of Decorin Leads to Accelerated Glomerular Lesions of Diabetic Nephropathy (perfused)



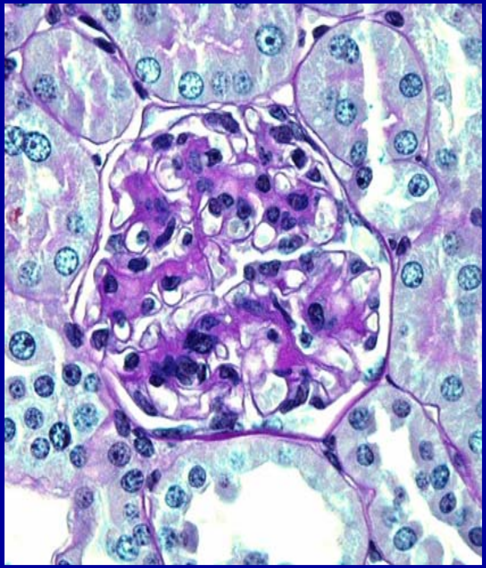
Diabetic (10-12 m)

Wild type



Diabetic (10-12 m)

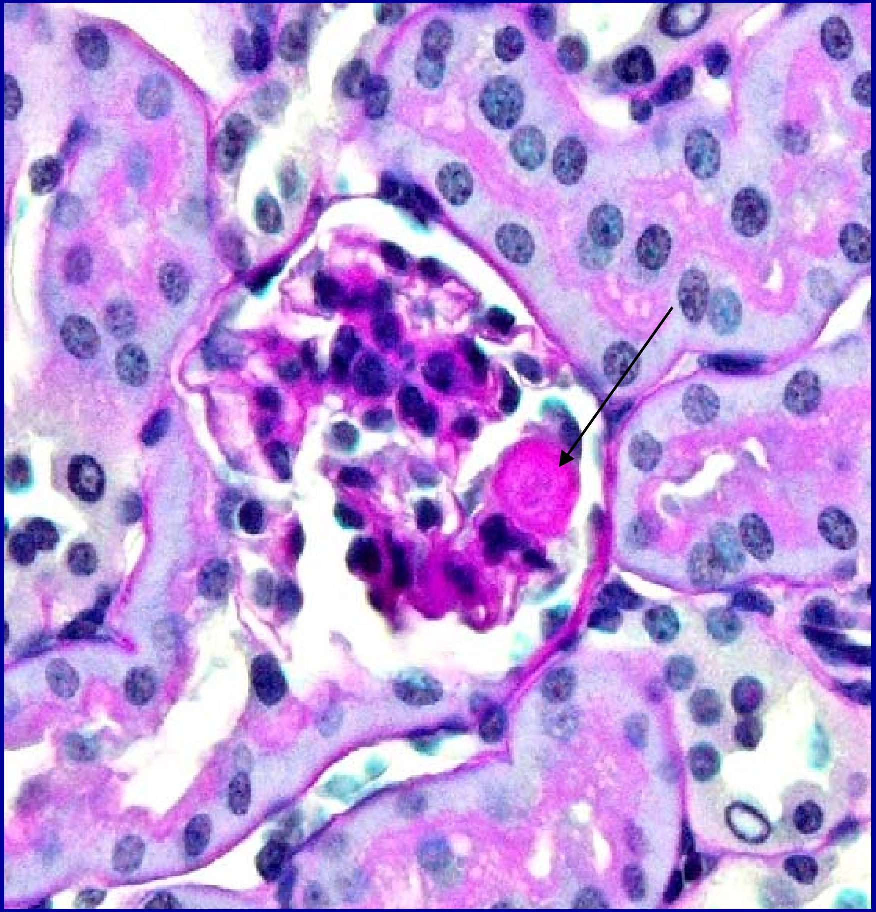
Dcn +/-



Diabetic (10-12 m)

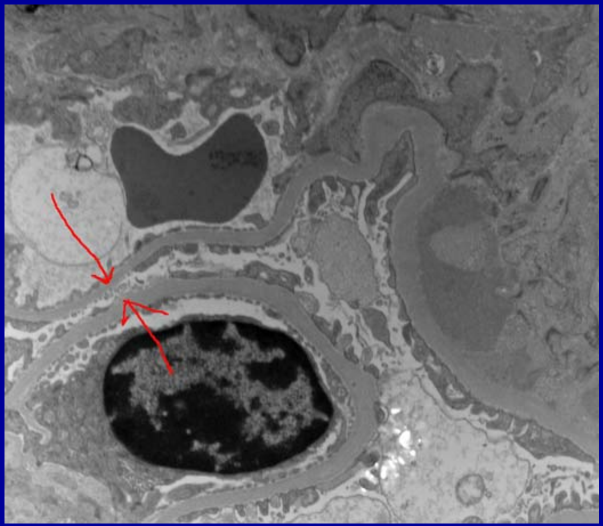
Dcn -/-

Nodular Lesions Observed in Decorin KO Diabetic Mice



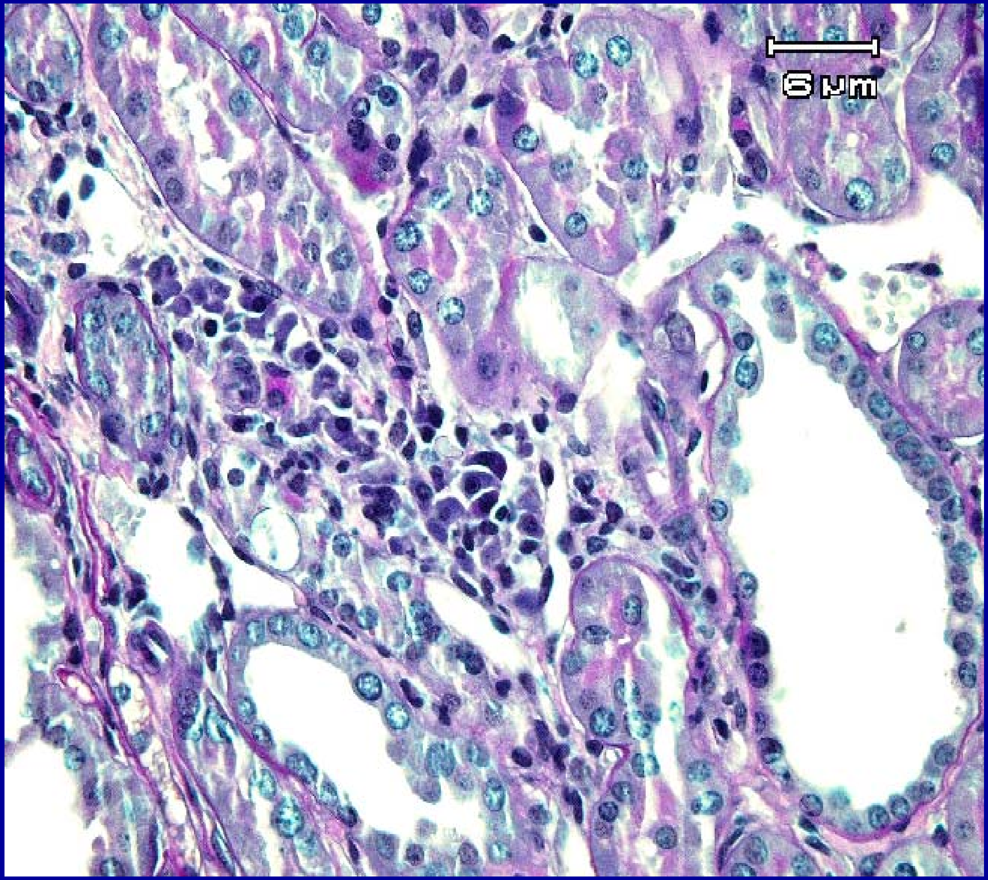
GBM Thickness and Podocyte Effacement Observed in Decorin Deficient Diabetic Mice

Normal
Foot processes
+/+ normal mouse



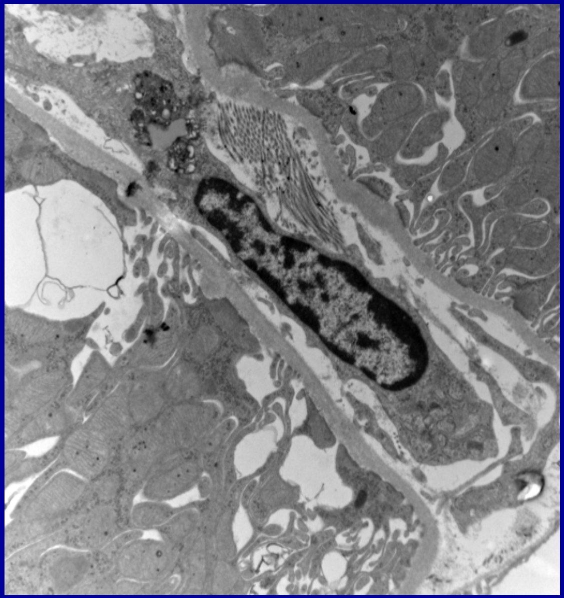
Denuded and thickened GBM
Dec +/- diabetic mouse

Loss of decorin leads to tubulointerstitial lesions in decorin KO diabetic mice

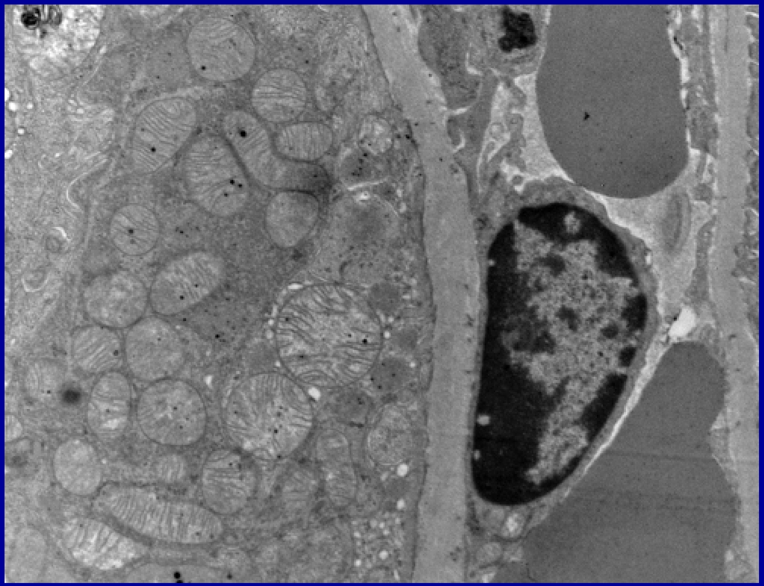


Interstitial cell infiltrate, tubular atrophy, and tubular dilation in decorin KO diabetic mouse

Loss of Decorin leads to TBM Thickening in Diabetic Mice



Normal TBM in
+/+ Non-Diabetic Mouse



Thickened TBM in
Decorin +/- Diabetic Mouse

Summary

- Prolonged diabetes using a low-dose STZ protocol in C57BL/6J mice leads to stable diabetes for 10-12 months
- Prolonged diabetes in wild type mice leads to a small increase in albuminuria and increased plasma creatinine
- Prolonged diabetes in wild type mice leads to progressive mesangial matrix expansion with GBM thickening
- Prolonged diabetes in wild type mice leads to TBM thickening without marked tubulointerstitial lesions

Summary

- Loss of decorin (Dcn -/-) in diabetic mice is associated with excess mortality by 12 months of diabetes. Much of the excess mortality is in mice with increased albuminuria and increased plasma creatinine
- Loss of decorin (Dcn -/-) in diabetic mice leads to an accelerated course of diabetic nephropathy characterized by increased albuminuria and increased plasma creatinine
- Loss of decorin allele leads to severe diabetic glomerulopathy with diffuse mesangial matrix expansion, occasional nodular expansion and GBM thickening
- Loss of decorin in diabetic mice leads to tubulointerstitial lesions with atrophic tubules and TBM thickening
- Decorin is likely to be an endogenous protective agent in the diabetic kidney

Ongoing Consortium studies

- Attempt to identify cause of excess mortality in decorin KO diabetic mice
- Finalize quantitation of degree of glomerular and tubulointerstitial disease in each group

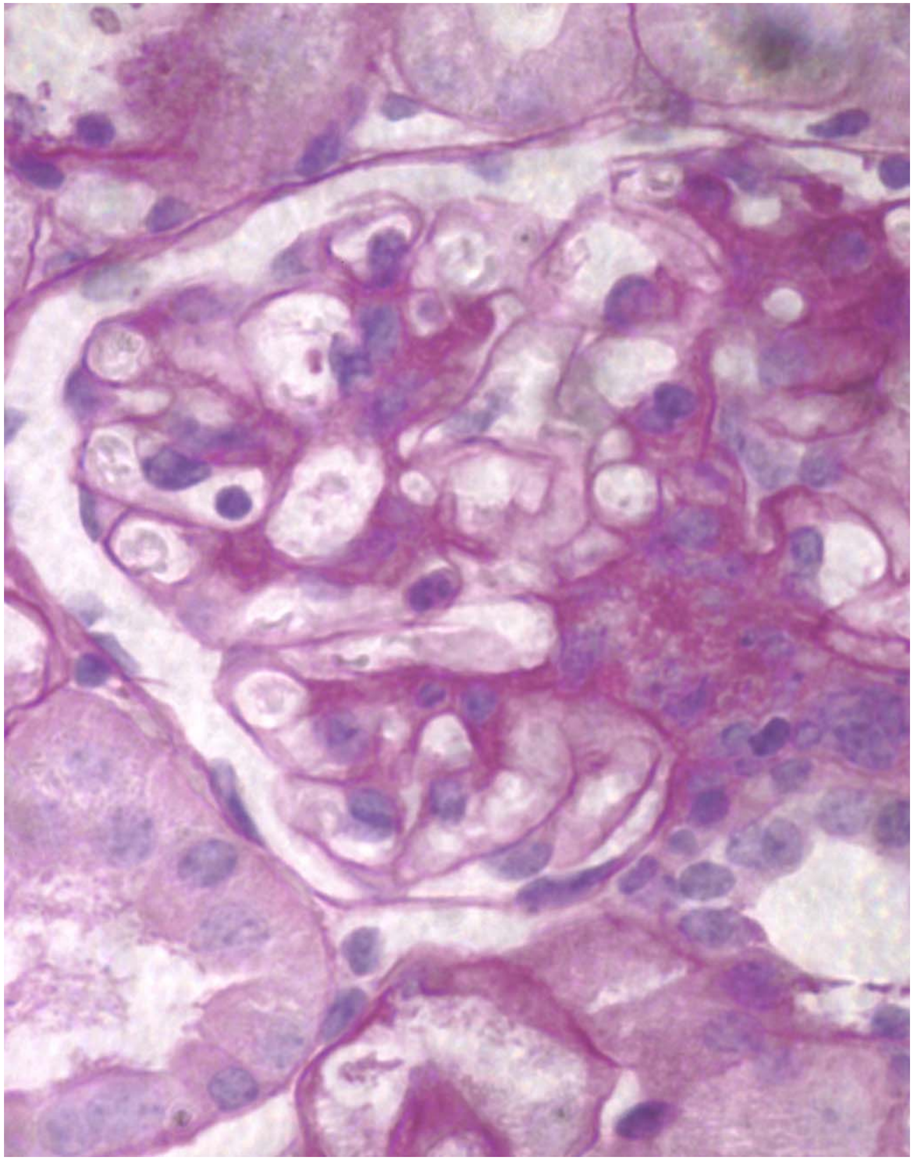
  -perfusion-fixation, EM analysis, morphometrics
- Assess the role of decorin in Akita mice-possibly more rapid onset of severe nephropathy
- Assess decorin deficiency in diabetic LDL-receptor KO mice
- ***Decorin KO diabetic mice did not develop excess retinopathy (performed by Tim Kern)***
- ***Decorin KO diabetic mice to be sent to Michigan for neuropathy phenotyping***

flk1RAGEtg with Low Dose STZ

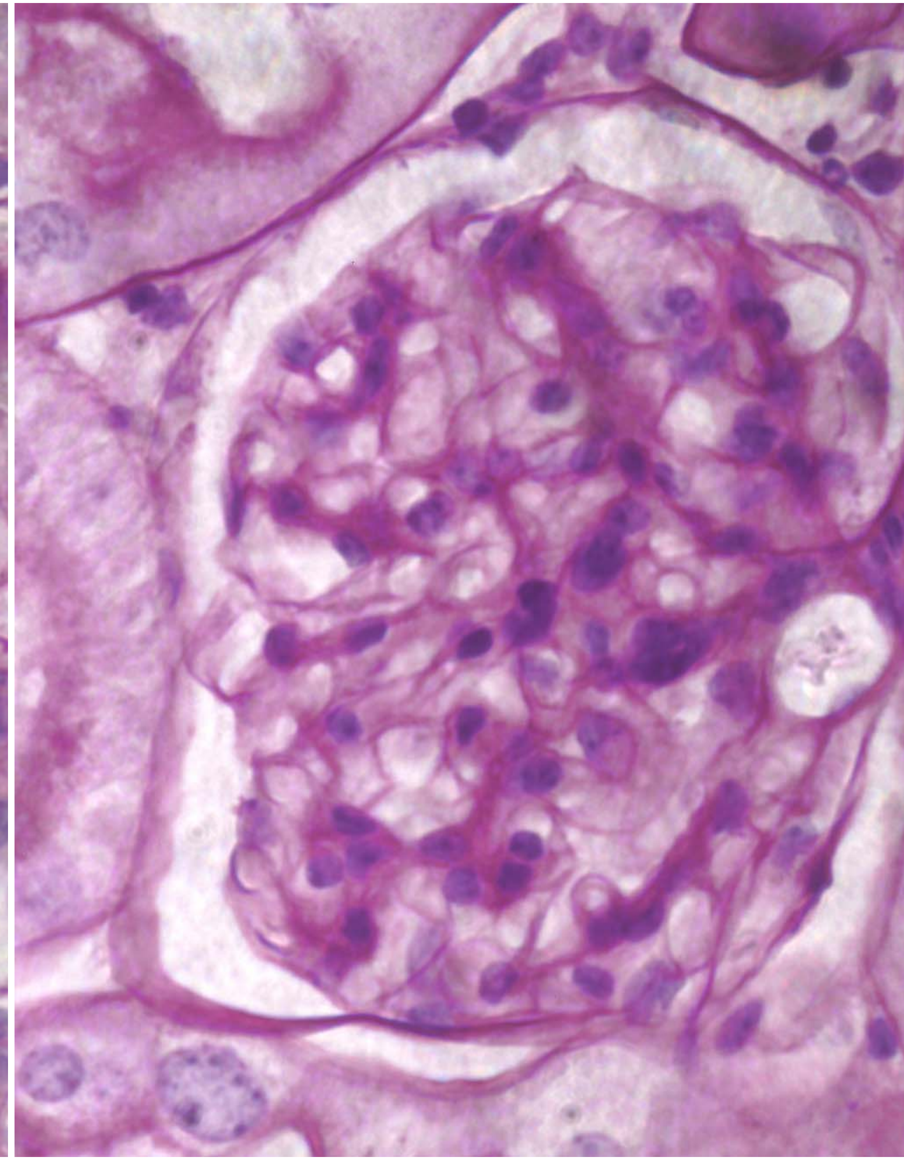
20W_RAGE_
STZ

	mouse ID#	genotype	sex	DOB	blood glucose (mg/dL)	body weight (gram)	16 hr urine (ml)	alb concentration	16 hr albumin	creatinine2	alb/cre2
1	1	T	F	5/10/2004	600	26.4	22	0.2	4.0	0.006	28.3
2	3	T	F	5/10/2004	514	27.5	15	1.8	27.6	0.014	135.8
3	4	T	F	5/10/2004	515	27.7	17	0.9	14.6	0.006	149.3
4	5	T	F	5/10/2004	600	27.8	8	2.3	18.6	0.015	152.5
6	9	T	M	5/10/2004	600	34.1	45	1.6	70.3	0.003	537.5
8	13	T	F	5/10/2004	489	28.7	30	1.0	30.1	0.005	186.5
9	16	T	M	5/10/2004	529	29.6	47	1.6	72.9		
10	18	T	F	5/11/2004	600	30.6	22	2.0	44.1	0.015	130.6
11	20	T	M	5/10/2004	451	24.9	76	0.8	62.2	0.005	171.3
12	23	T	F	5/11/2004	600	25.2	26	1.4	37.1	0.006	247.7
13	26	T	F	5/11/2004	600	24.4	24	0.7	16.4	0.003	201.3
5	8	W	M	5/10/2004	600	37.7	60	0.4	21.7	0.002	163.2
7	11	W	M	5/10/2004	581	28.3	83	2.3	187.5	0.004	595.5

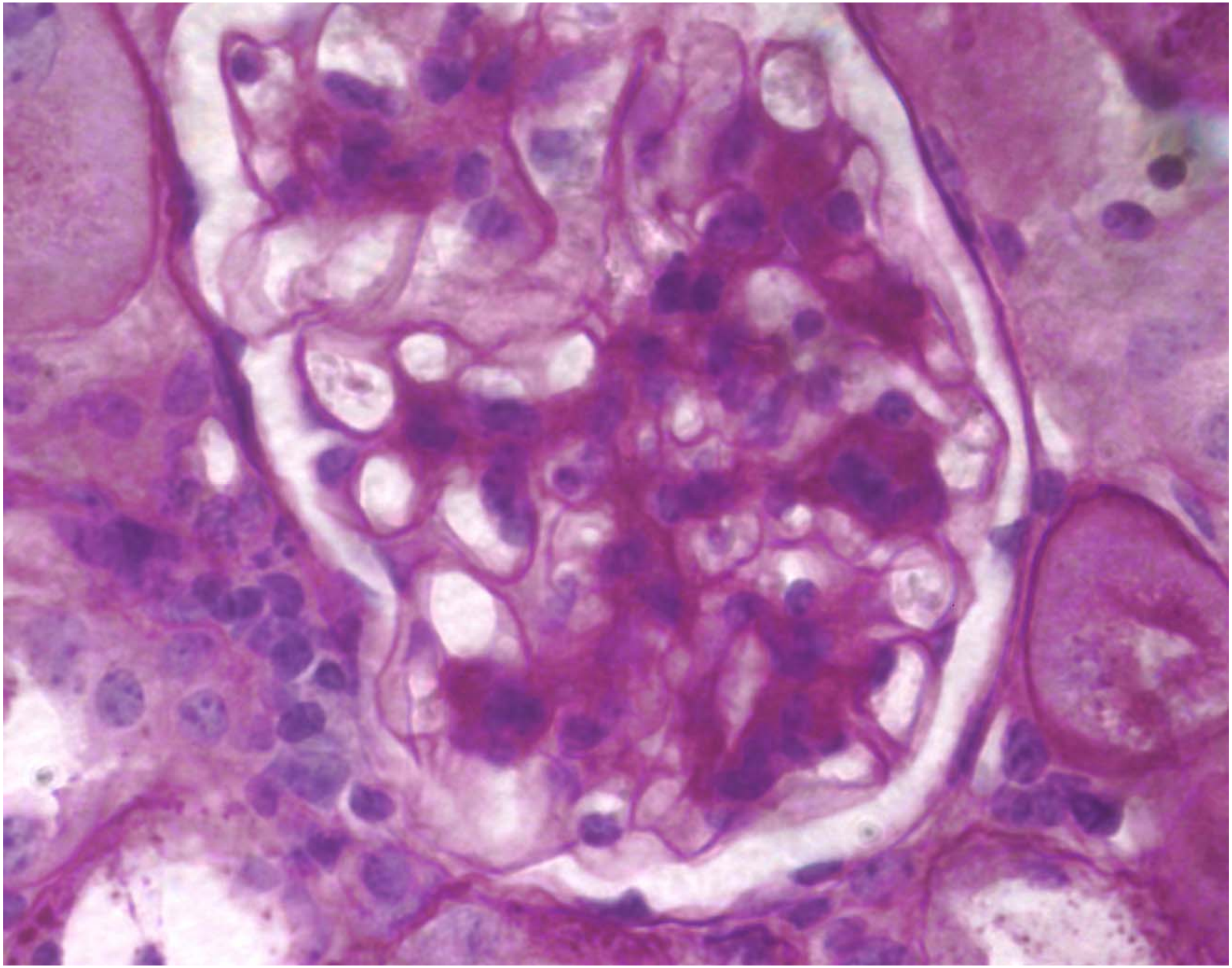
flk1RAGE STZ 20 wk



WT STZ 20 wk



flk1RAGE20a STZ 20 wk



flk1RAGE: Ongoing/Proposed Work

- *Animals in Pipeline for*
  - Extend baseline characterization
  - Send eyes to Tim Kern (Retinopathy)
  - Send samples/animals to Eva Feldman (Neuropathy) & Firouz Daneshgari (Uropathy)
  - flk1RAGEtg X Akita
- *Planned/Proposed Experimental Models*
  - flk1RAGEtg shift to DBA background
  - provide mice for Cardiovascular Consortium groups

New Model Development

- Tie1-Glut1 x Akita
- Nphs2-Glut1 tg x Akita

- *gGT-CD36 tg x Akita*

2004 Publications

Diabetes, 53:784, 2004

Molecular Profiling of Diabetic Mouse Kidney Reveals Novel Genes Linked to Glomerular Disease

Katalin Susztak,[1] Erwin Böttinger,[1,2] Akiva Novetsky,[1] Dan Liang,[1] Yanqing Zhu,[3] Emilio Ciccone,[3] Dona Wu,[1,2] Stephen Dunn,[3] Peter McCue,[4] Kumar Sharma[3]

Kidney International, 65:---, 2004

Utility of endogenous creatinine clearance as a measure of renal function in mice

STEPHEN R. DUNN, ZHONGHUA QI, ERWIN P. BOTTINGER, MATTHEW D. BREYER, and KUMAR SHARMA

Dorrance Hamilton Research Laboratories, Division of Nephrology, Department of Medicine, Thomas Jefferson University, Philadelphia, Pennsylvania; Division of Nephrology, Department of Medicine, Vanderbilt University, Nashville, Tennessee; and Division of Nephrology, Department of Medicine, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York

04-PLME-RA-0120R1 Revised Manuscript Submitted October 12, 2004; PLoS Medicine

Multiple Metabolic Hits Converge on CD36 Scavenger Receptor as Novel Mediator of Tubular Epithelial Apoptosis in Diabetic Nephropathy

Katalin Susztak1,2, Emilio Ciccone3, Peter McCue4, Kumar Sharma3, ‡, Erwin P. Böttinger1, ‡

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