Mouse Models of Diabetic Nephropathy

Mount Sinai / Jefferson / Einstein / Minnesota

Erwin Böttinger, PI

Kumar Sharma, Co-PI

Group Members

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

Work-In-Progress

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

- *Experimental Models*
  Bottinger (MSSM)
    completed
    - db/db C57BLKS longitudinal, standardized phenotyping
    - Ins2-Akita longitudinal, standardized phenotyping

    finalizing
    - flk-1 RAGEtg with low dose STZ

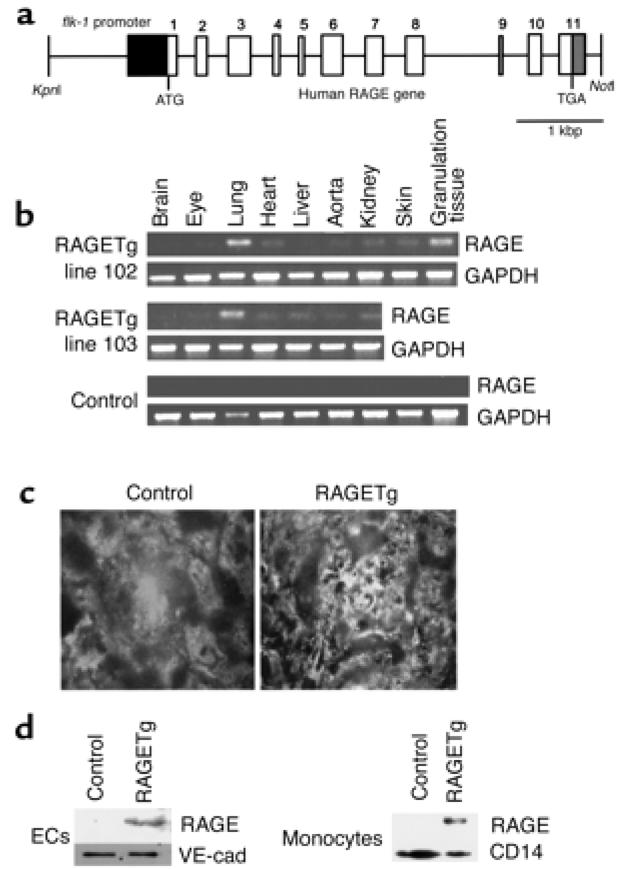
    ongoing
    - flk-1 RAGEtg with Ins2-Akita
    - Cd2ap+/- with STZ
    - Cd2ap+/- with Ins2-Akita

  Kumar Sharma (TJU)
    finalizing
    - Decorin knockout with low dose STZ

- *Phenotyping Cores*
  HPLC creatinine… Kumar Sharma/Steve Dunn (TJU)
  Genomics…. Erwin Bottinger (MSSM)

Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice
Yasuhiko Yamamoto and Hiroshi Yamamoto;
J Clin Invest, 2001



DIABETES INDUCTION

inducible nitric oxide synthase (iNOS) under the control of the insulin promoter (iNOSTg)

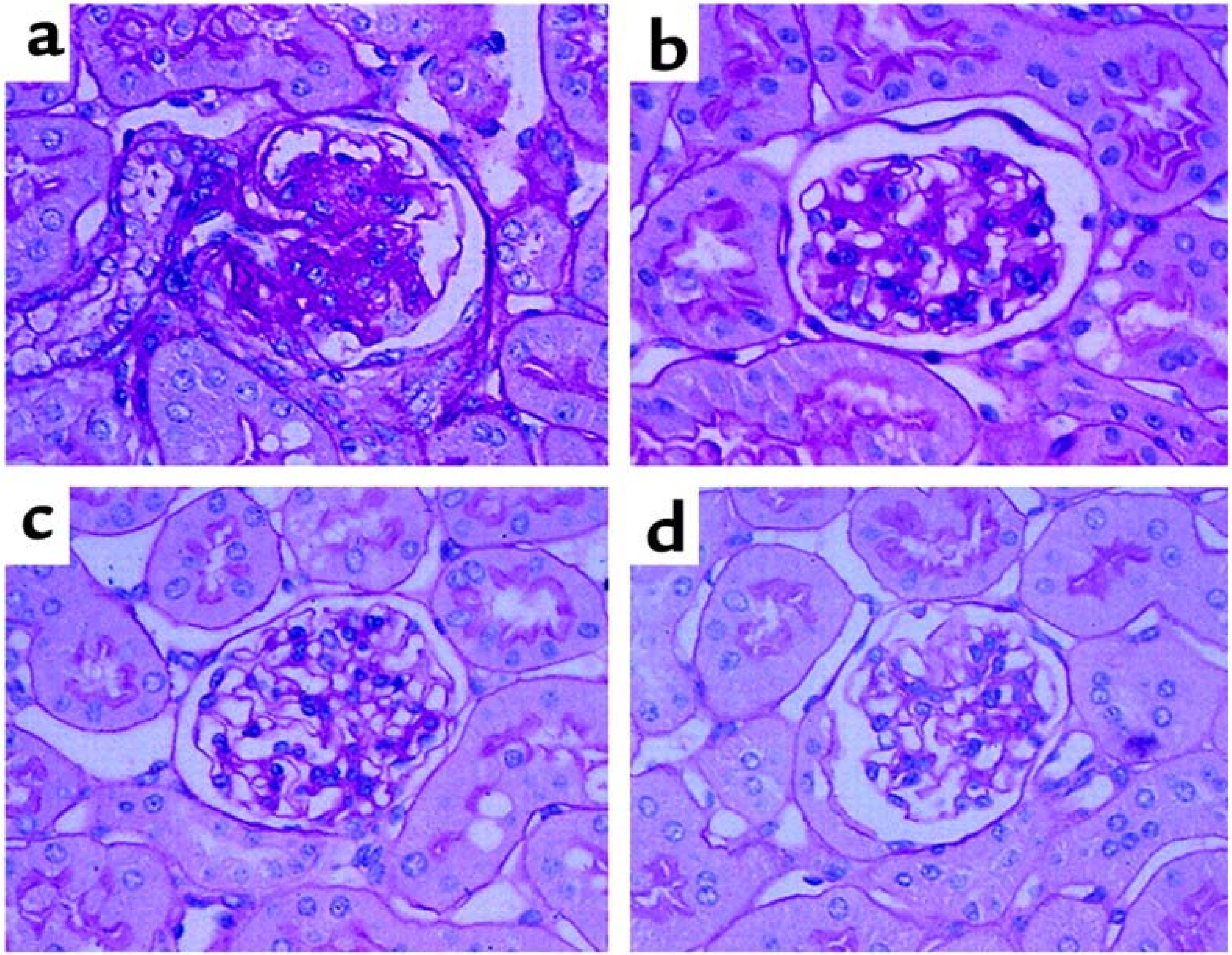
Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice

Yasuhiko Yamamoto and
J Clin Invest, 2001

Four months old

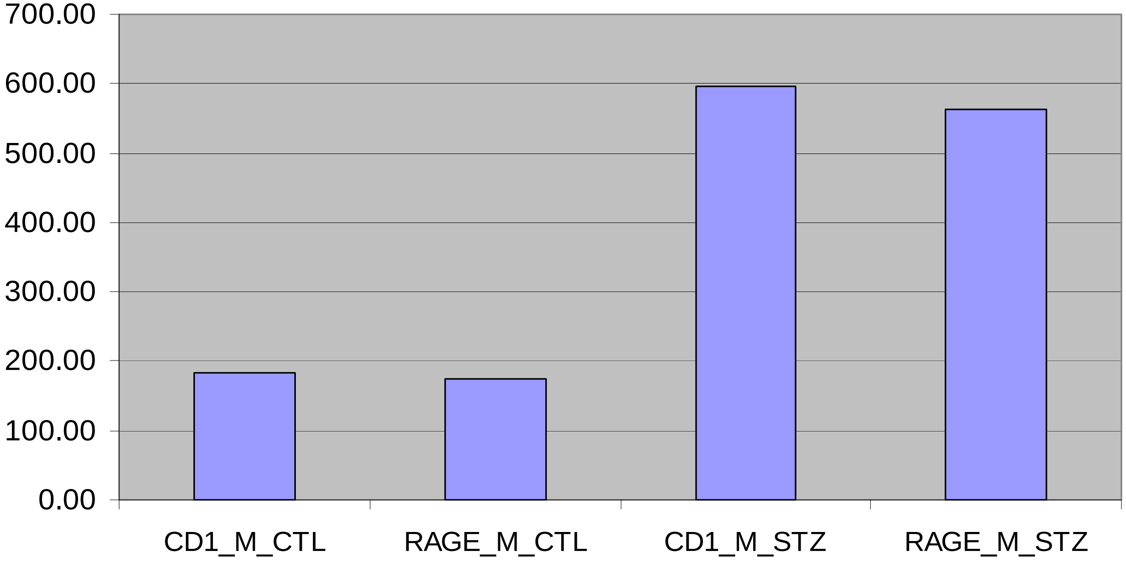
a) DM+ RAGE+
b) DM+ RAGE-
c) DM- RAGE+
d) DM- RAGE-



Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

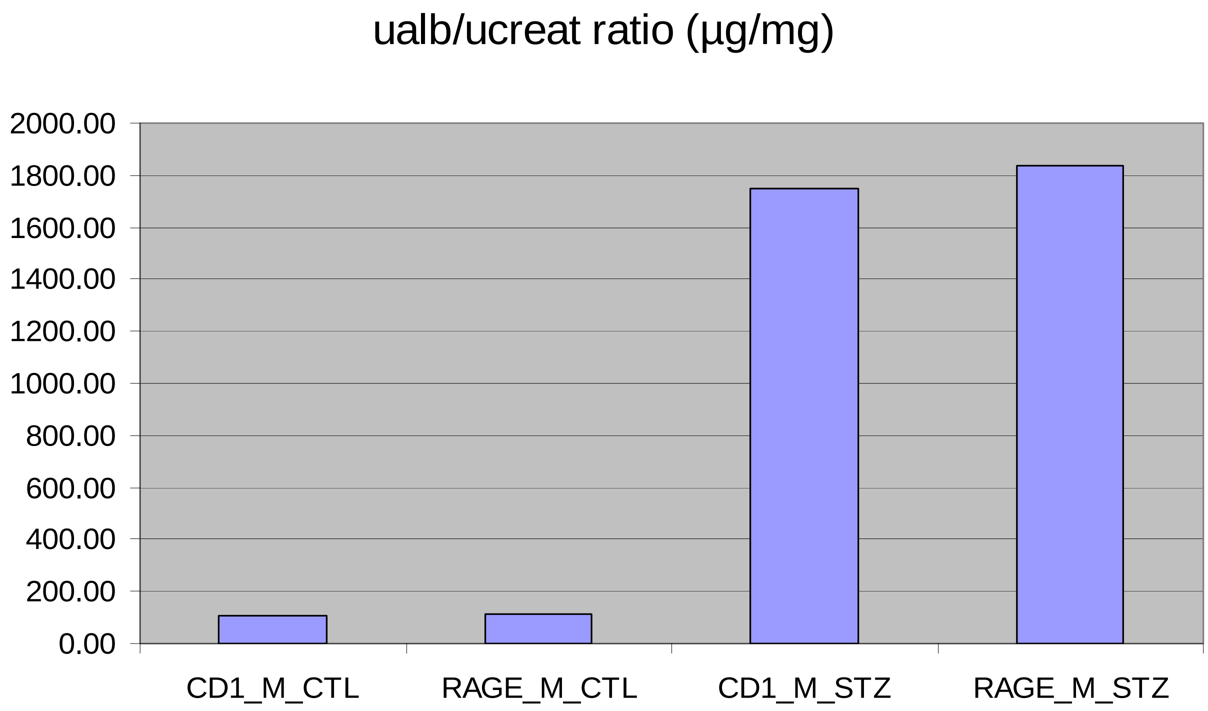
Experimental Design
- Female and Male mice
- CD1 inbred
- STZ 8 wk
- Sac 20 wk

blood glucose (mg/dL)



Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

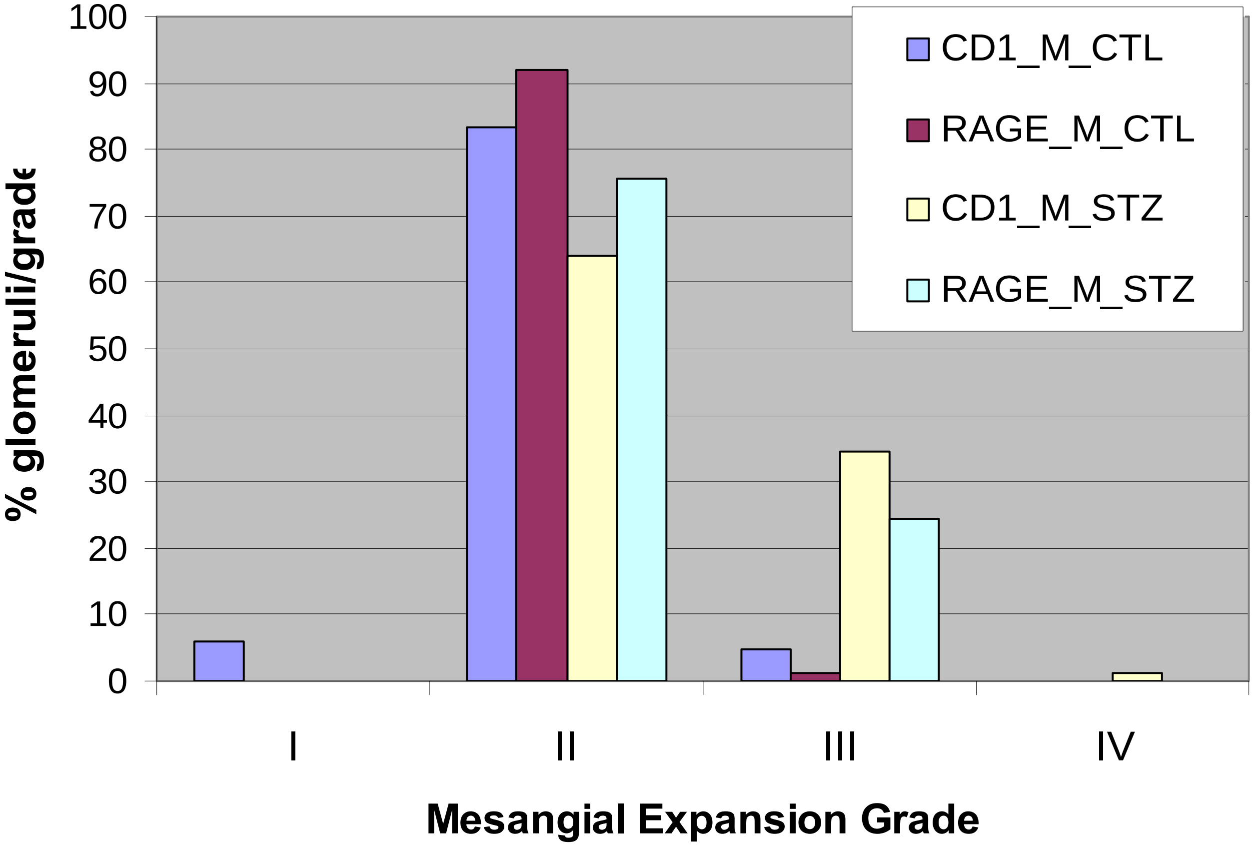
Experimental Design
- Female and Male mice
- CD1 inbred
- STZ 8 wk
- Sac 20 wk



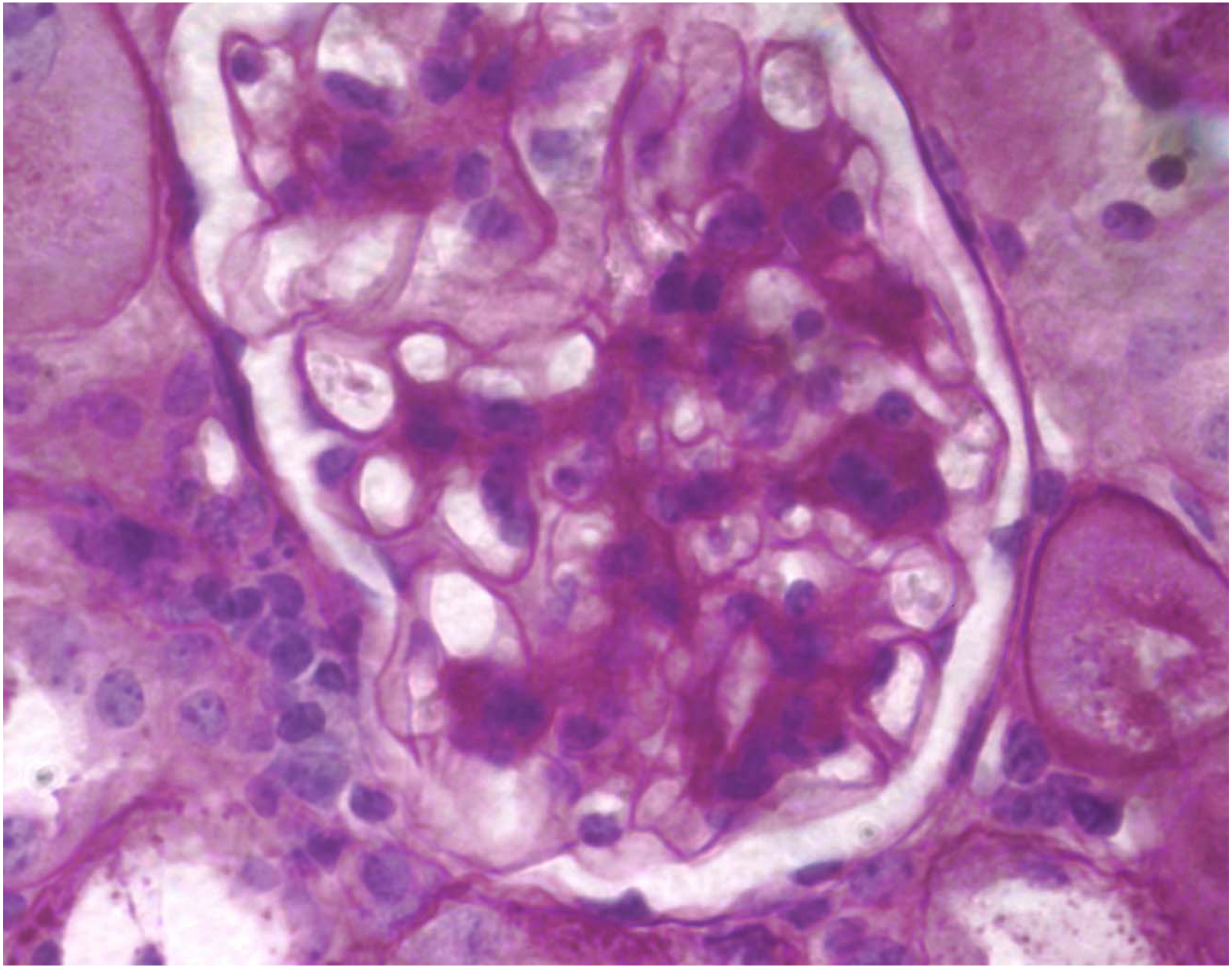
Low Dose STZ in flk-RAGE tg [CD1-tg(Kdr-Ager)]

Experimental Design
•Male mice
•CD1 inbred
•STZ 8 wk
•Sac 20 wk

Pathology Report
•No nodular lesion
•No tub.int. lesions
•No vascular leseions

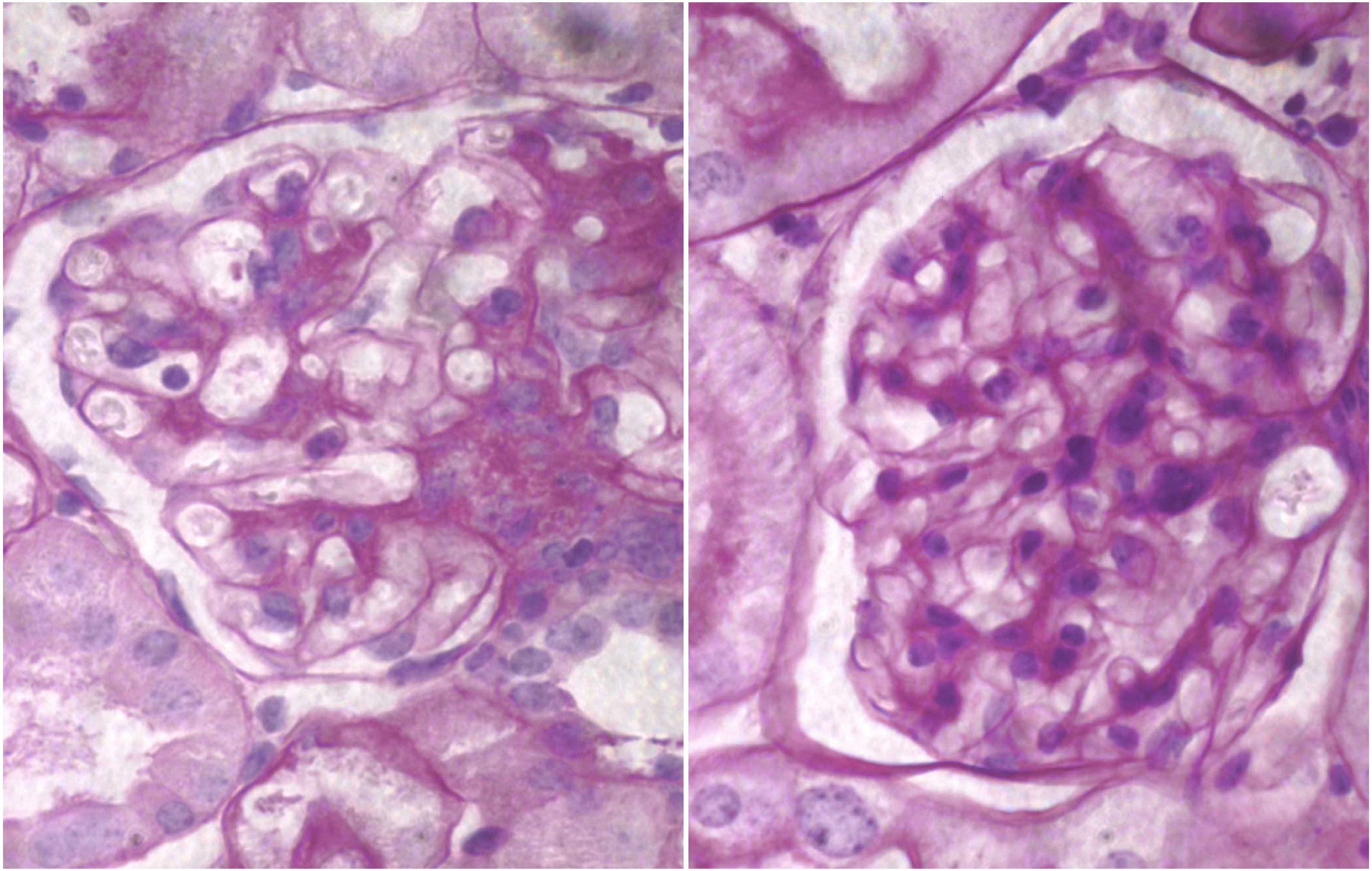


flk1RAGE20a STZ 20 wk



flk1RAGE STZ 20 wk

WT STZ 20 wk



Flk-RAGE tg Plans 2005/2006

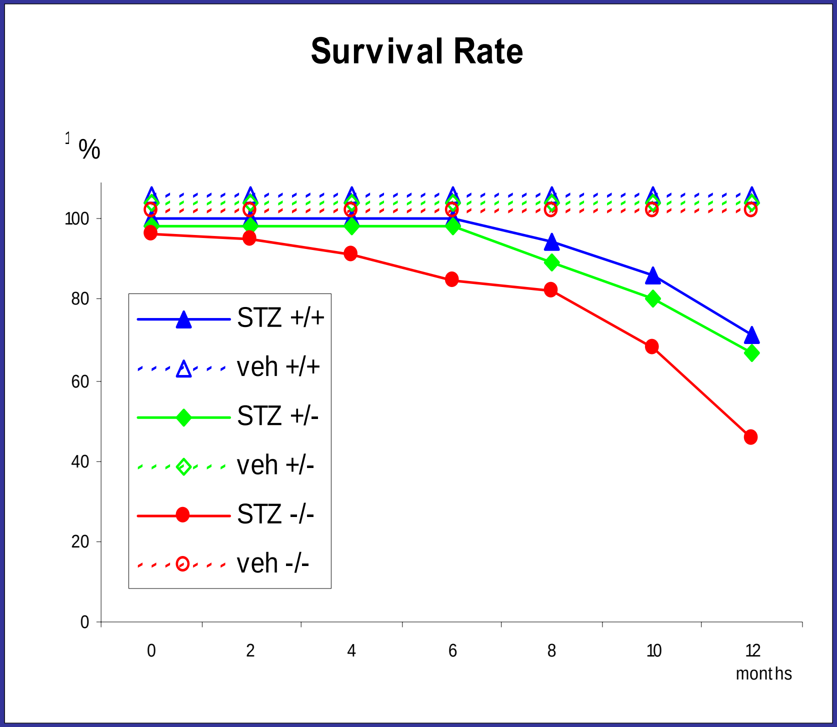
- flk-RAGE tg [CD1-tg(Kdr-Ager)]
  - Cohorts
    - Ins2-Akita backcross to CD1
    - Ins2-Akita x flk-RAGE in CD1
    - follow to age
    - Open end

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

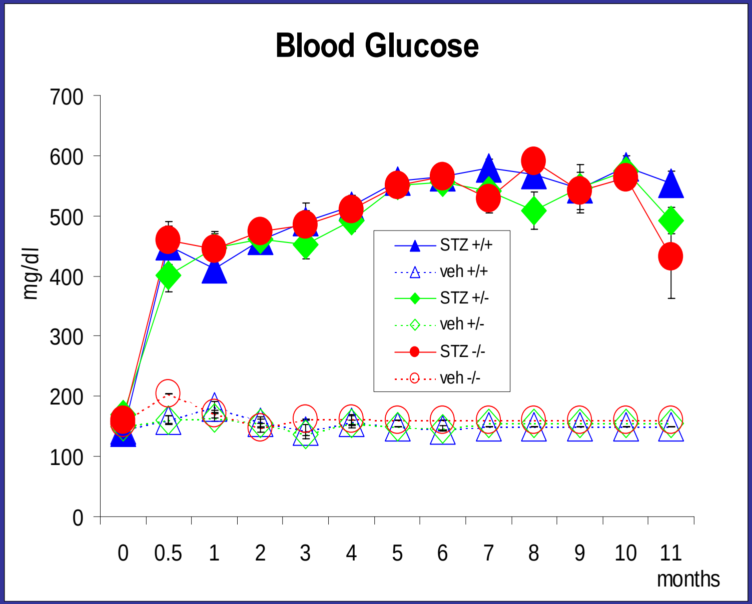


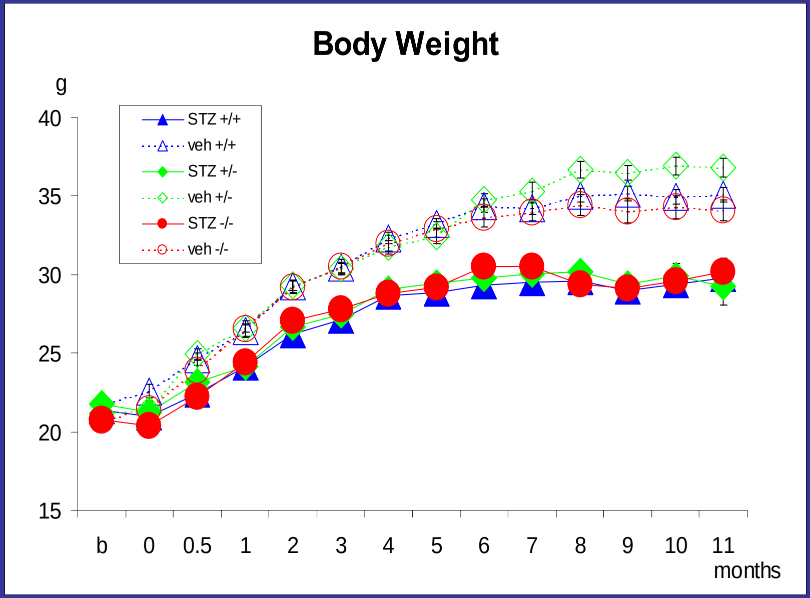
- Over ½ (12/22) of decorin KO diabetic mice died spontaneously by 12 months of diabetes
- Wild type diabetic mice had less mortality by 12 months of diabetes (4/14)
- The diabetic mice that died were characterized by increased plasma creatinine and increased albuminuria compared to mice that survived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

Clinical Parameters of Diabetic Mice that Died Spontaneously

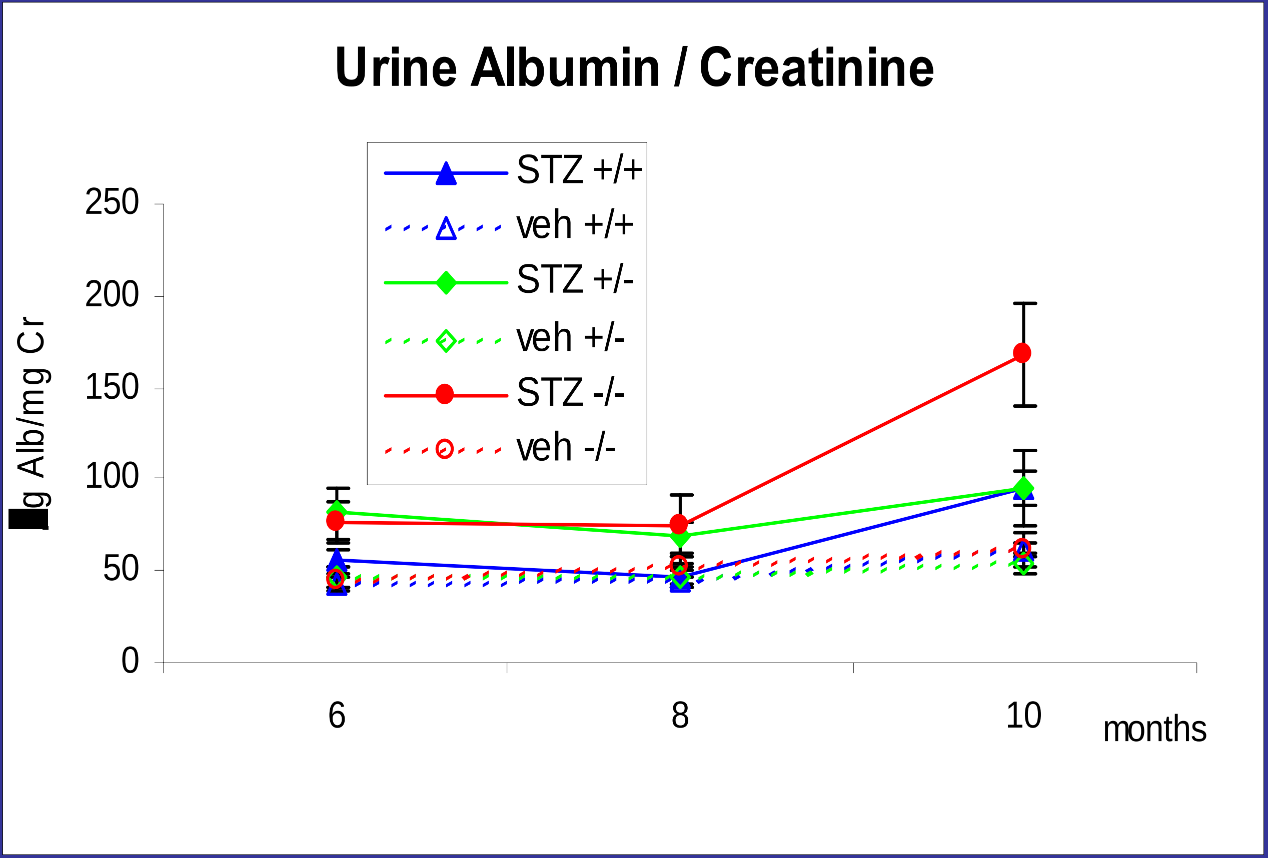
	Diabetic Mice (survived, n=30)	Diabetic Mice (died <12 m, n=21)
Body wt (g)	29.17 ± 0.65	29.96 ± 0.57
Blood gluc (mg/dl)	499 ±32.3	553.85 ±17.0
STZ dose	333 ±24.6	314 ±24.7
Urine alb/creat	98.8 ±11.7	115.52 ±21
Plasma Creat (mg/dl)	0.22 ±0.01	0.27 ±0.02

Blood Glucose and Body Weight in Diabetic Mice

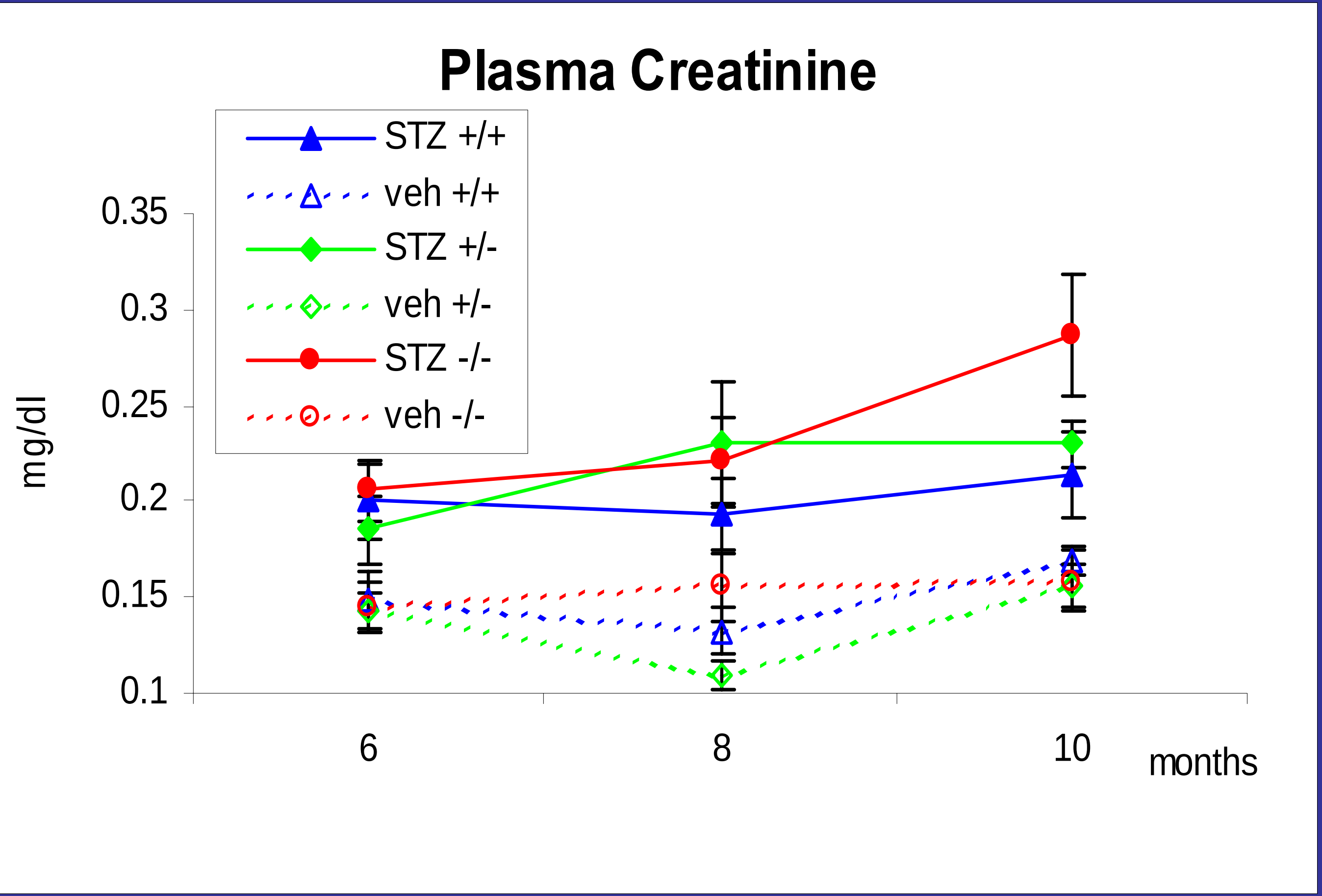




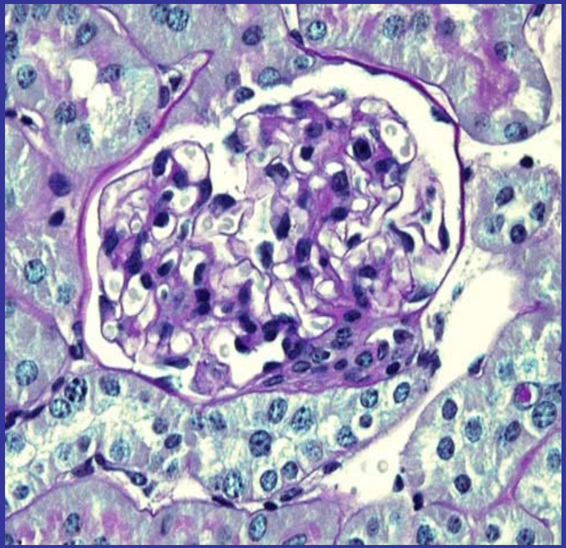
Loss of Decorin Results in Increased Diabetic Albuminuria



Loss of Decorin Contributes to Renal Dysfunction in Diabetes

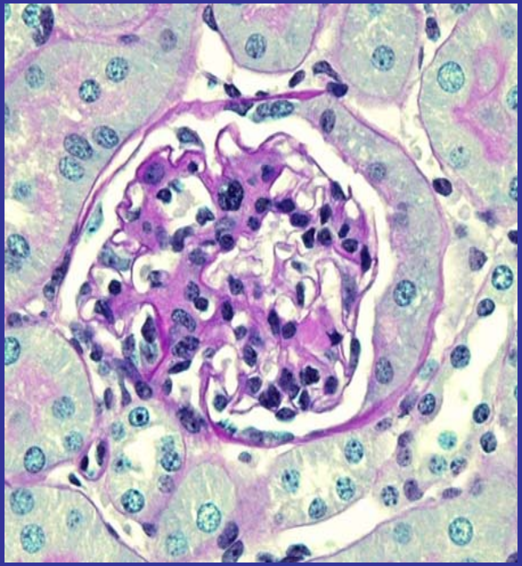


Loss of Decorin Leads to Accelerated Glomerular Lesions of Diabetic Nephropathy (perfused)



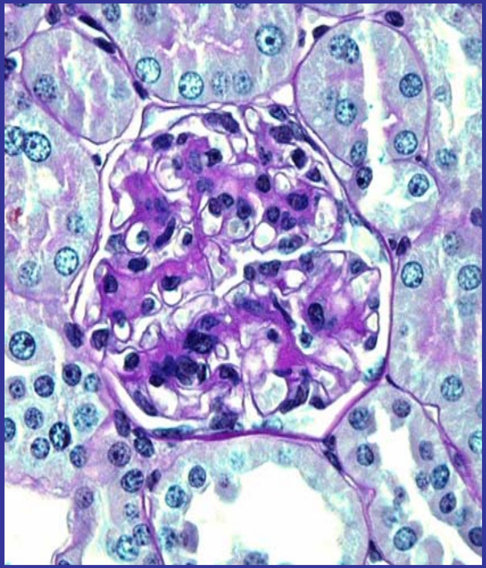
Diabetic (10-12 m)

Wild type



Diabetic (10-12 m)

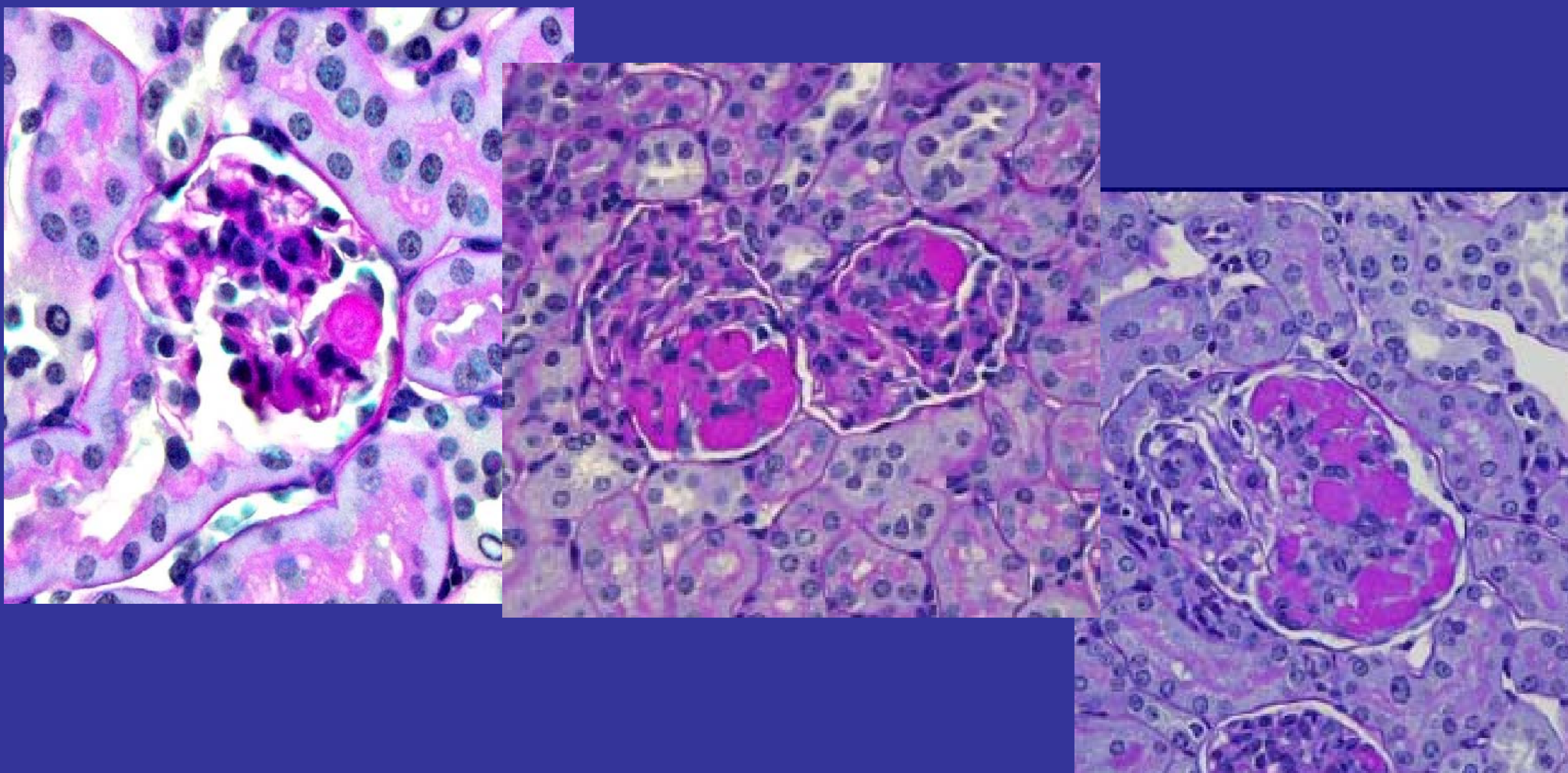
Dcn +/-



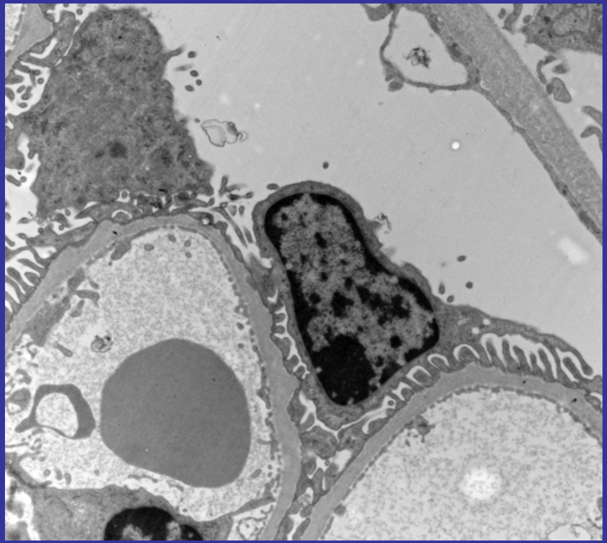
Diabetic (10-12 m)

Dcn -/-

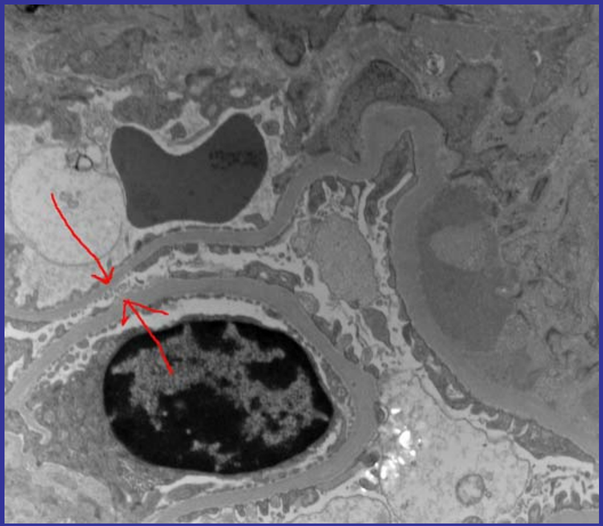
Nodular Lesions Observed in Decorin KO Diabetic Mice



GBM Thickness and Podocyte Effacement Observed in Decorin Deficient Diabetic Mice

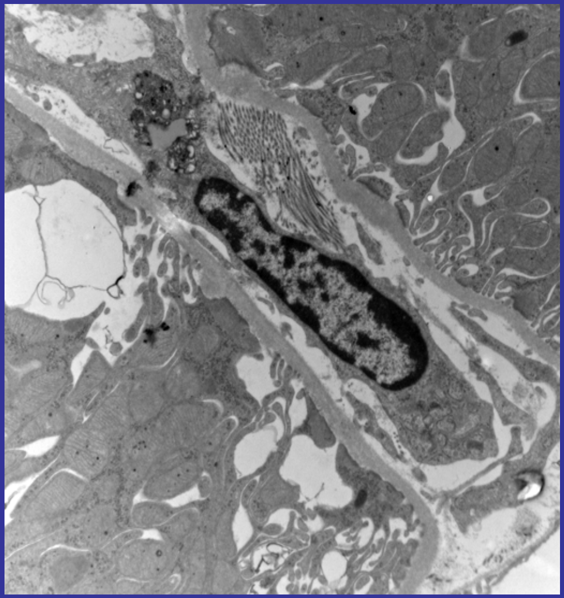


Normal
Foot processes
+/+ normal mouse

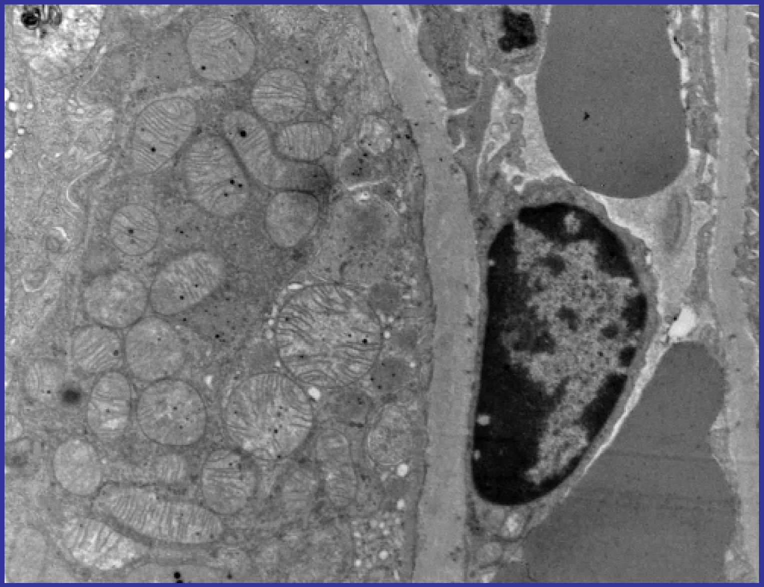


Denuded and thickened GBM
Dec +/- diabetic mouse

Loss of Decorin leads to 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

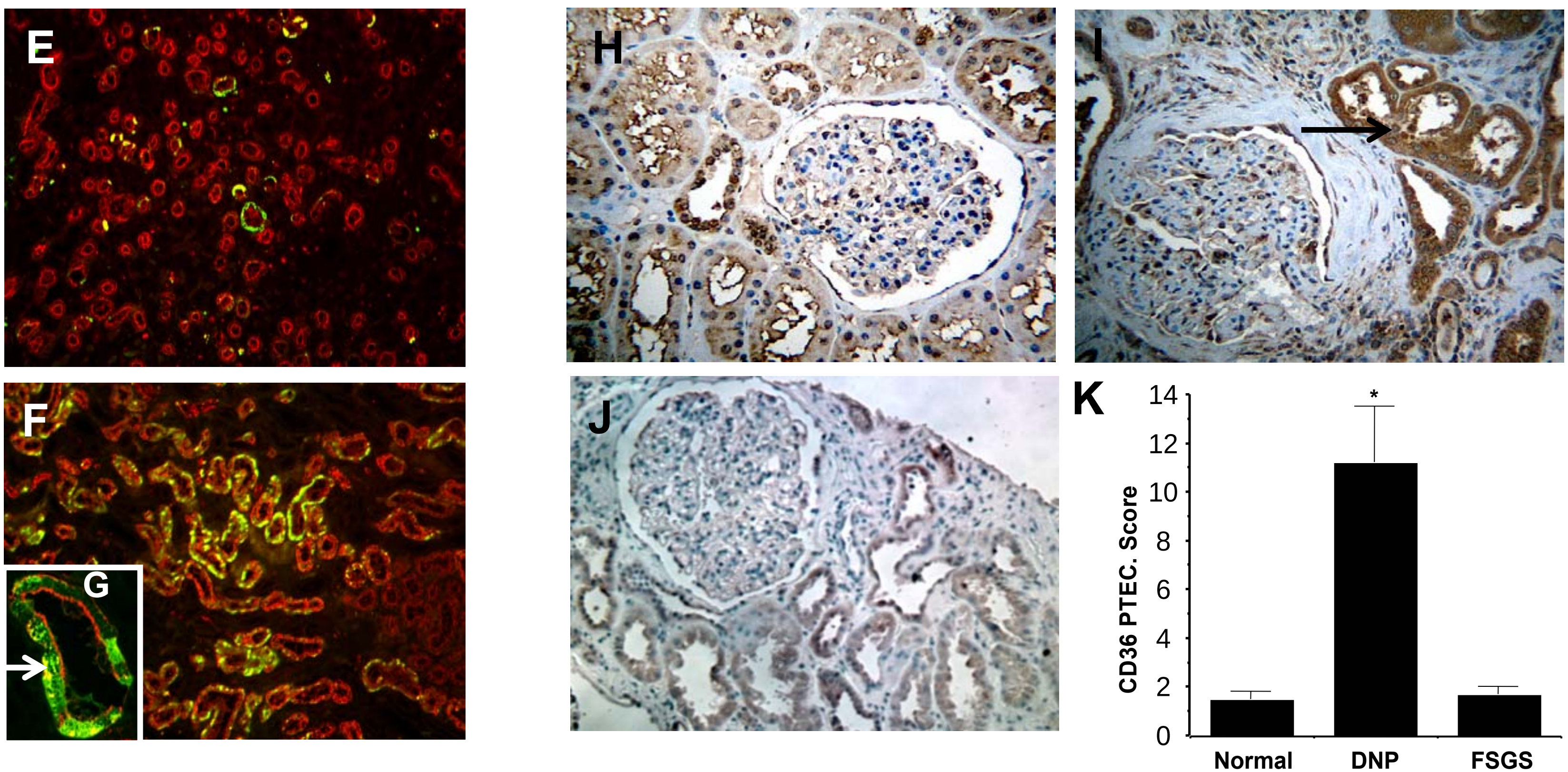
Ongoing Consortium studies

- 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***

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

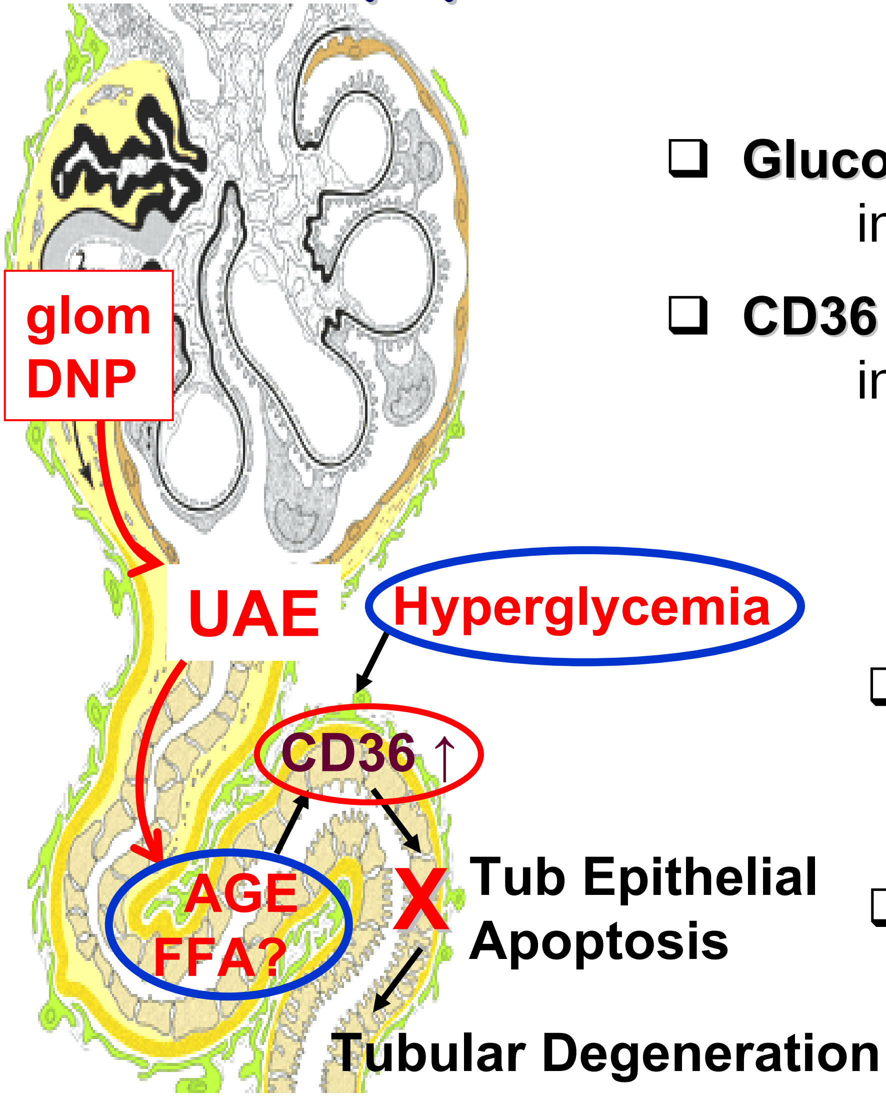
Katalin Susztak[1,2], Emilio Ciccone[3], Peter McCue[4], Kumar Sharma[3*], Erwin P. Böttinger[1*]



NOVEL CONCEPT II:

Tubular Apoptosis and Tubular Degeneration

MSSM
MOUNT SINAI SCHOOL OF MEDICINE



- **Glucose stimulates CD36** expression in PTECs in vitro
- **CD36** expression is increased in in **prox tubules** in **human DNP**
- **AGE** and **FFA** may induce **tubular epithelial apoptosis** through **CD36**-dependent signaling
- **Tubular epithelial apoptosis** may underlie **tubular degeneration**

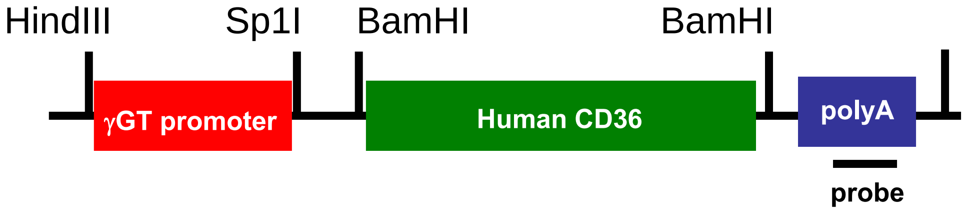


Figure 4. Schematic representation of GGT/CD36 targeting vector

db/db C57BLKS/J Molecular Phenotyping

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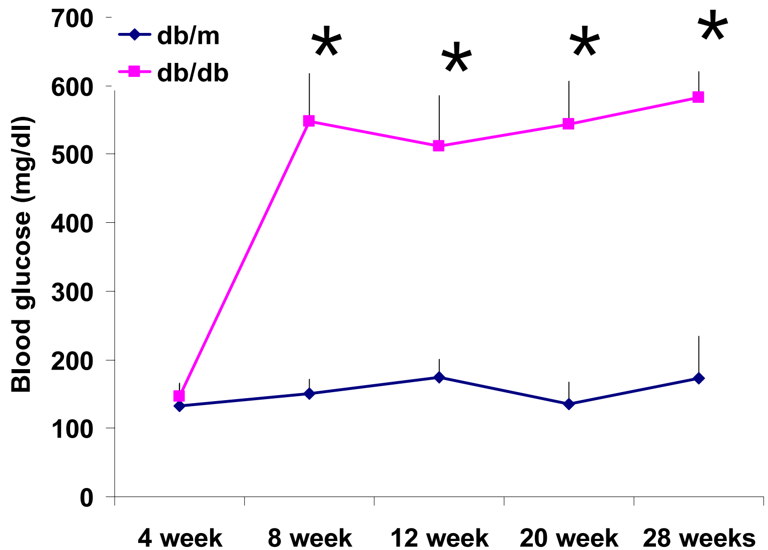
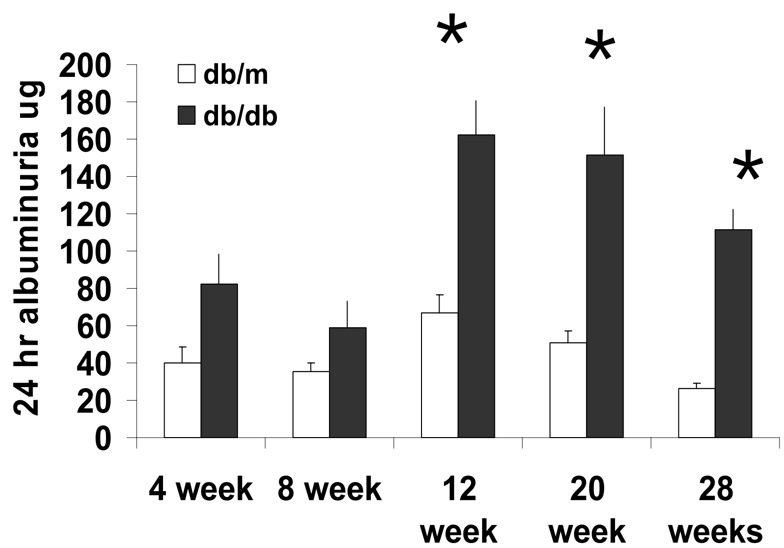
Blood Glucose (BG)



Urinary Albumin Excretion (UAE)



Glomerular and tubular RNA preparations

↓

MOE430 whole genome profiling

Glomerular Molecular Fingerprints in Diabetic Kidneys

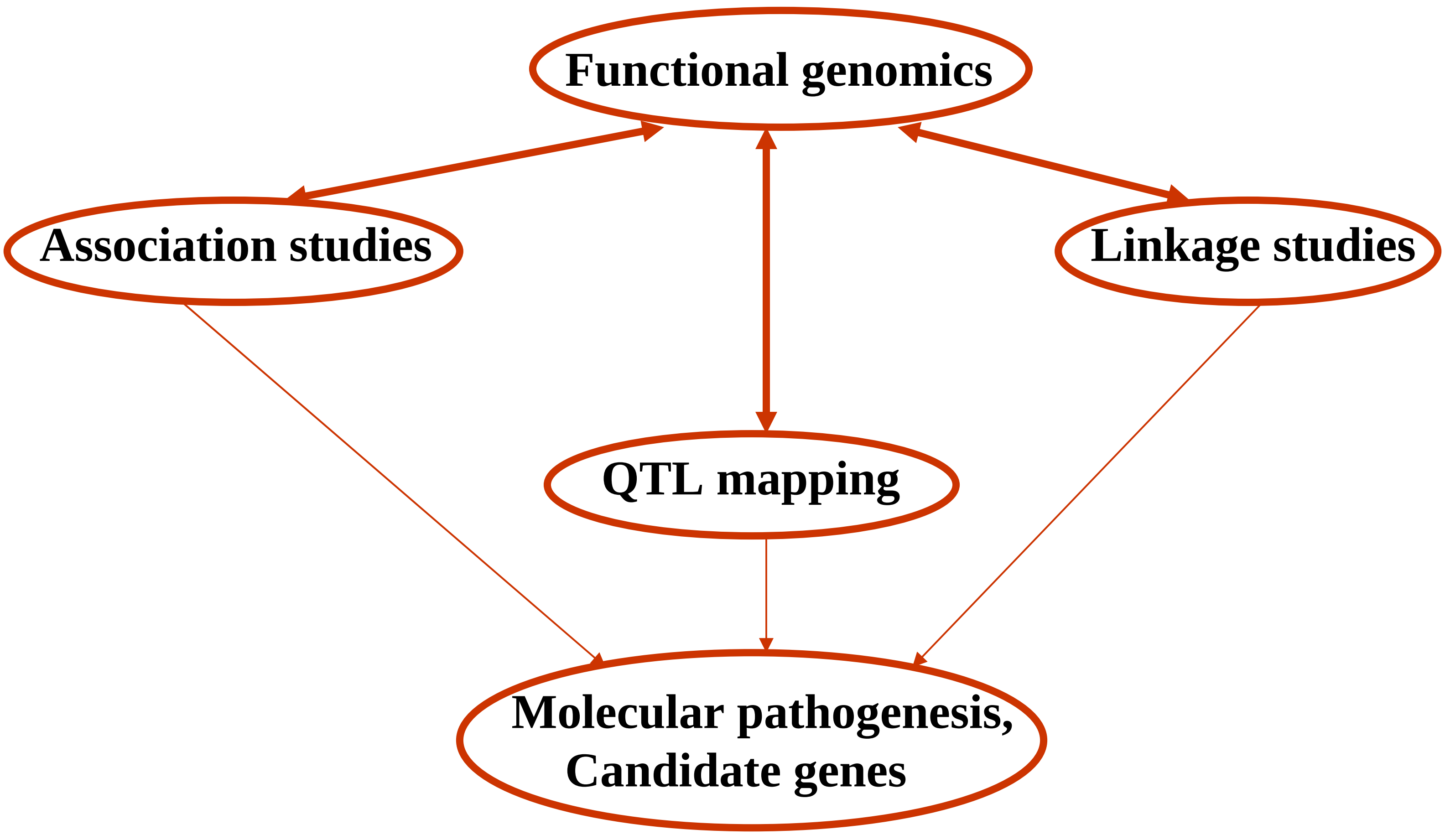
Purity- and Quality-Controlled Glomerular Preps (3 mice pooled)
AFFYMETRIX 430 Mouse Whole Genome Arrays
Standardized Data Analysis Algorithms

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

Genetic/Genomic/Physiologic Data Integration



Project Goals

- **to integrate functional genomics data (i.e. microarrays gene expression datasets, mutagenesis screen results) with genetic and phenotype/disease traits association data.**

- **to generate cross-reference for experimentally significant genes and the genetic basis of QTLs in rodents and humans (*genetical genomics* approach).**

- **to provide genetic and (patho)physiologic annotation of putative candidate genes for kidney (or other) disease and prediction of their functions and biological association.**

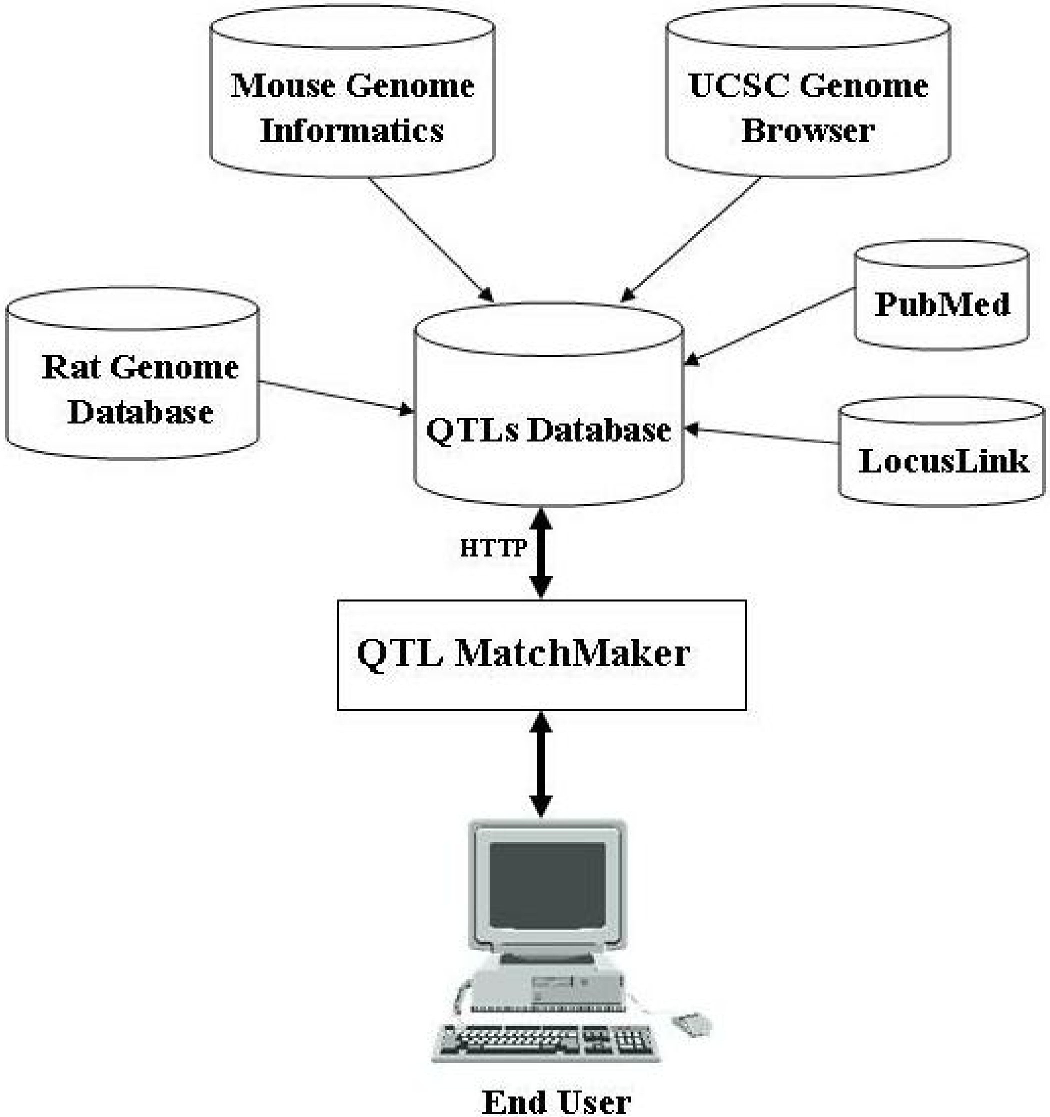

Mouse Genome Informatics
UCSC Genome Browser
PubMed
Rat Genome Database
QTLs Database
LocusLink
HTTP
QTL MatchMaker
End User

Table 1: QTL Database Statistics

Species	Distinct Traits	Total QTL/Linkage Groups	Average Interval Length (bp)
Mus musculus	323	1475	39864556
Ratus norvegicus	81	629	40498069
Homo sapiens	109	143	24336058

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QTL MATCHMAKER

Understanding the molecular basis of quantitative variation has the potential to explain numerous phenotypes, including common human diseases, such as asthma, atherosclerosis, diabetes, hypertension, and obesity. The goal of QTLs (Quantitative Trait Loci) mapping is to identify and characterize the DNA sequences responsible for these conditions. Although it has been very difficult to conduct linkage studies in humans, QTLs have been mapped in inbred species: rodents, insects and plants. Despite this progress, finding the genes underlying quantitative traits remains a challenge.

QTL MATCHMAKER is a tool that addresses this problem. It allows for high throughput mapping of genes to known QTLs and comparative genomics analysis between Homo sapiens, Mus musculus, Rattus norvegicus. The QTL database integrates information from numerous web sites and publications.
QTL MATCHMAKER is designed to facilitate the analysis of large sets of data produced using genome-scale experimental approaches.

Choose organism: Mus musculus

Submit

| QTL Graphs | QTL Upload |

Feedback: kvstar@aecom.yu.edu

QTL MATCHMAKER is provided by Bottinger Lab, MSSM.

Compiled from a number of public databases.
Data are curated from MGD , RatMap , RGD , PubMed , OMIM ,and UCSC Genome Browser .
Powered by ***LIMS team*** @ Mount Sinai School of Medicine, 2004

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Organism Choosen: Mus musculus

Choose traits:

- All
- skull morphology
- Angiostrongylus costaricensis nematode susceptibility
- B-cell maturation defect
- B.burgdorferi-associated arthritis
- Babesia resistance
- CD4 T cell subset
- CD4 virgin T cell subset
- CD8 T cell subset
- CD8 T memory cell subset

Enter an accession number: Maximum input text is 50 characters.

Submit Reset Form

Batch search: data for multiple genes

Representative Report File

Accession number	Transcription Start (bp)	Transcription End (bp)	QTL Symbol	Trait	Chromosome	QTL Statistics	Reference	PubMed ID#
NM_009088	72874205	72944492	Igt-1	impaired glucose tolerance	6	4	Shrike, Diabetes; vol. 50; Aug 2001; pp 1943-1948	11473059

| **Home** |

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You may enter the Accessions numbers as a **list**. The input must be a text consisting of one Accessions number per line. You must select at least one check box from the **desired information** below.

Paste or Enter accession numbers:

Note: Maximum input text is 50 characters per row and maximum 200 accession numbers.

Organism chosen:

Mus musculus

Choose desired information:

☐ QTL Name	☐ Trait	☐ Chromosome	☐ Cytoband	☐ Alignment Start
☐ Alignment End	☐ Epidemiology	☐ QTL Statistics	☐ Reference	☐ PubMed ID

Submit | Reset Form

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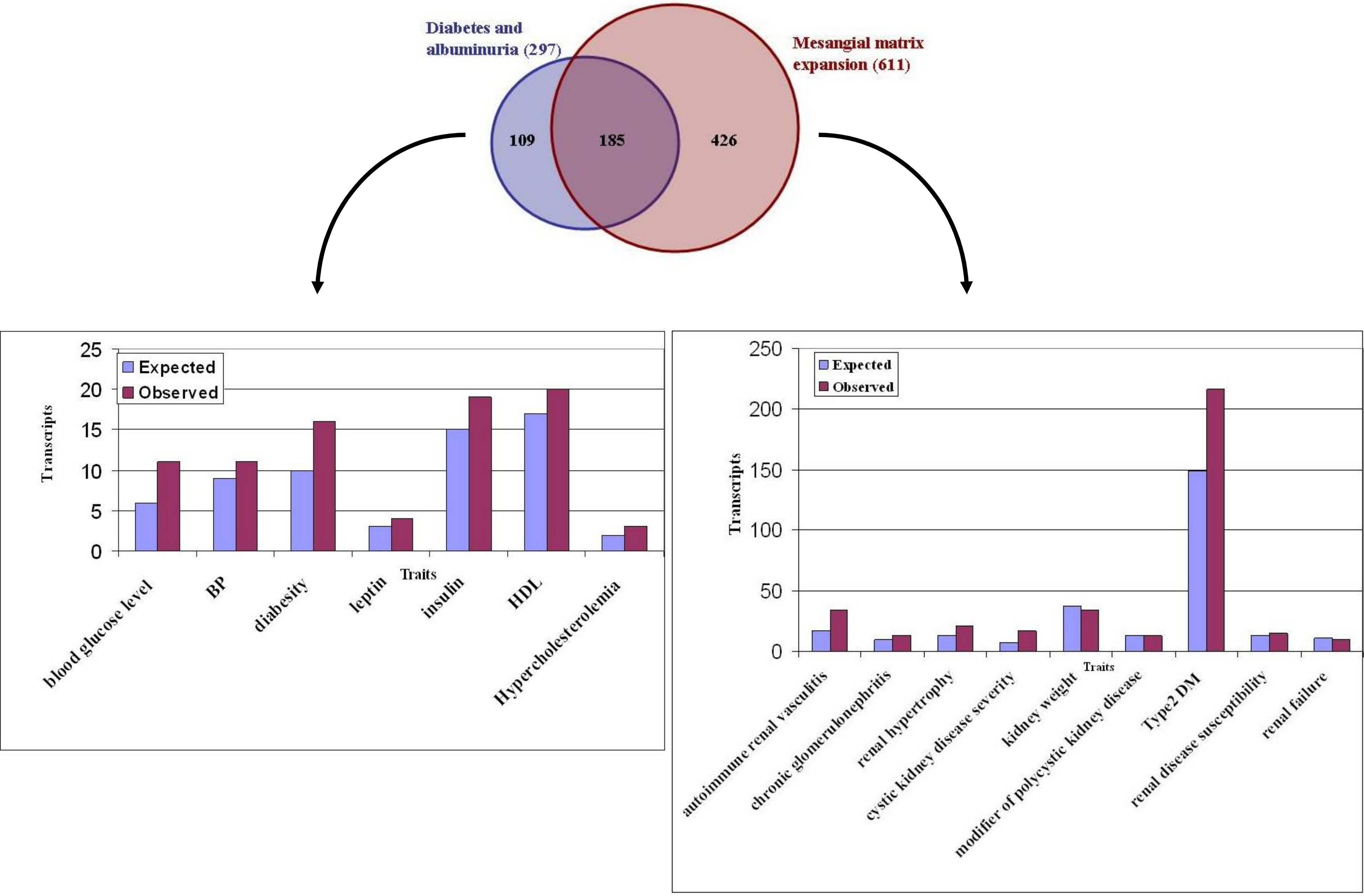


Diabetes and albuminuria (297)
Mesangial matrix expansion (611)
109
185
426
Expected
Observed
Transcripts
Traits
blood glucose level
BP
diabesity
leptin
insulin
HDL
Hypercholesterolemia
Expected
Observed
Transcripts
Traits
autoimmune renal vasculitis
chronic glomerulonephritis
renal hypertrophy
cystic kidney disease severity
kidney weight
modifier of polycystic kidney disease
Type2 DM
renal disease susceptibility
renal failure

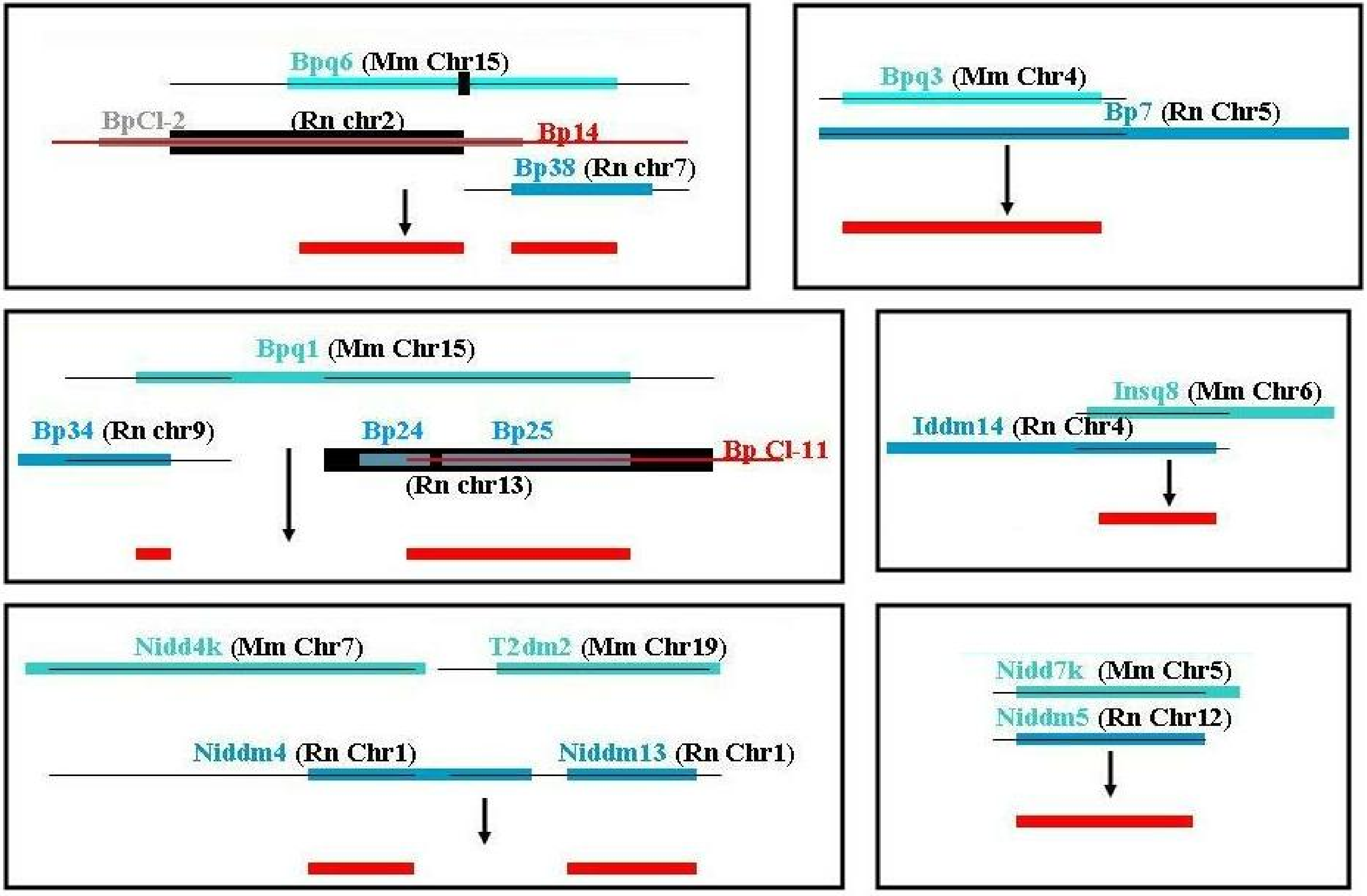
QTL MatchMaker application to the Mm. and Rn. orthologues of a SLE gene cluster from laser-captured glomeruli microarrays (samples from clinical biopsies of SLE patients (Peterson et al., 2004)).

Homo sapiens		**Mus musculus**				**Ratus norvegicus**			
Gene	**RefSeq**	**Mm Orthologue**	**QTL**	**Trait**	**Statistics**	**Rn Orthologue**	**QTL**	**Trait**	**LOD**
TGFBR3	NM_003243	NM_011578	Lmb2	lupus	4.5	NM_017256	Rf4	renal disease susceptibility	4.1
VDP	NM_003715	NM_019490	Lmb2	lupus	4.5	NM_019379	Rf4	renal disease susceptibility	4.1
AFP	NM_001134	NM_007423	Lmb2	lupus	4.5	NM_012493	Rf4	renal disease susceptibility	4.1
CDK2	NM_001798	NM_007659	Lmb4	lupus	5	NM_199501	Uae2 3	urine albumin level early onset	2.4
IQGAP2	NM_006633	NM_016721	Lmb3	lupus	3	NM_012609	Vetf2	vascular elastic tissue fragility	4.5
ANXA6	NM_001155	NM_013472	Lbw8	lupus	P<0.005	NM_024156	Vetf2	vascular elastic tissue fragility	4.5
MAPK9	NM_002752	NM_016961	Lbw8	lupus	P<0.005	NM_017322	Pur2	proteinuria	5.5
SERPINE1	NM_000602	NM_008871	Lbw3	lupus	P<0.001	NM_012620	Eau2	uveitis severity	
ADORA2A	NM_000675	NM_009630	Lmb4	lupus	5	NM_053294	Cia1	collagen-induced inflammation	
HNRPH1	NM_005520	NM_021510	Lbw8	lupus	P<0.005	NM_080896	Pur2	proteinuria	5.5
PPARD	NM_006238	NM_011145	Lbw1	lupus	P<0.01	NM_013141	Cia1	collagen-induced inflammation	
ADORA1	NM_000674	NM_009629	Mbis 1	M. bovis-induced SLE	4.5	NM_017155	Uae1 1	urine albumin level	5.7
ADORA1	NM_000674	NM_009629	Sle8	SLE susceptibility		NM_017155	Uae1 1	urine albumin level	5.7
TSN	NM_004622	NM_011650	Sle8	SLE susceptibility		NM_021762	Pur1	proteinuria development	18.1
CDK2	NM_001798	NM_016756	Sle12	SLE susceptibility	3.54	NM_199501	Uae2 3	urine albumin level early onset	2.4
ACCN4	NM_018674	NM_183022	Sle7	SLE susceptibility		NM_022234	Uae2 5	urine albumin level early onset	3.5
IGFBP2	NM_000597	NM_008342	Sle7	SLE susceptibility		NM_013122	Uae2 5	urine albumin level early onset	3.5
PPARD	NM_006238	NM_011145	Sles1	SLE suppressor		NM_013141	Cia1	collagen-induced inflammation	

Renal and metabolic traits loci used in the syntenic approach to *in silico* QTL fine mapping.

Trait (Mus musculus)	**QTL**
Blood glucose level	7 (Bglu1, Bglu2, Fbg1-2, Fglu, Fglu2, Hypn)
Bood pressure	6 (Bpq1, Bpq2, Bpq3, Bpq4, Bpq5, Bpq6)
Diabesity	9 (Dbsty1-4, Dob2-4, Dob7, Dob9)
Leptin	2 (Elpt, Lepq1)
Insulin	1 (Fpli)
HDL	11 (Hdl1-4, Hdlq2-5, Hdlq 10-12)
Hypercholesterolemia	1 (Hypch)
Autoimmune renal vasculitis	2 (Arvm1-2)
Chronic glomerulonephritis	1 (Cgnz1)
Renal hypertrophy	1 (Crhq1)
Cystic kidney disease severity	1 (Ckds)
Kidney weight	7 (Kwq1-7)
Modifier of polycystic kidney disease	2 (Mopkd1-2)
Type 2 diabetes mellitus	21 (Nidd1-6, Nidds, Niddk1-7, Niddn1-4, T2dm1-3)
Renal disease susceptibility	2 (Rendc, Rends/Wbw1)
Renal failure	2 (Renf1-2)
Glomerulonephritis resistance	1 (Lprm3)

In silico redefining of QTL borders based on cross-species comparison. Syntenic blocks are in black, QTL are in color and red represents the predicted QTL borders.



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