



University of Michigan/Nephropathy

Modifications in Mouse Models to Enhance Diabetic Nephropathy (DN)

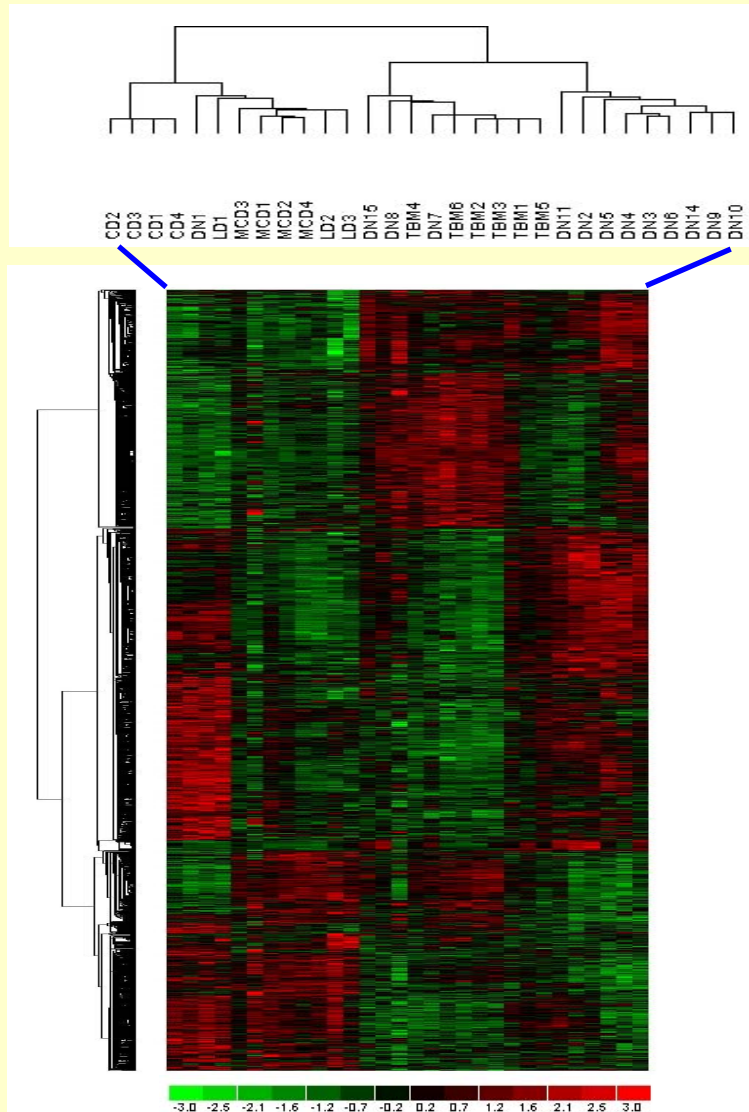
- ***Current murine models fail to show human-like DN because they fail to replicate glomerular and tubulointerstitial gene expression changes that occur in humans with progressive DN.***
- ***Replication of the critical transcriptomic profiles of patients with progressive DN should induce progressive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



Gene-expression map of human renal disease

- Human renal biopsy consortium
 - Micro-dissected glomerular and tubulo-interstitial compartments
 - Linear amplification
 - Hybridization of RNA on human Affymetrix chips HG_U133 A+B
 - ~39.000 transcripts / 6.000 splice variants
 - 313 samples hybridized
- Diabetic Nephropathy
 - Early DN (II-III) protocol biopsies: 106 Pima Indians
46 arrays
 - Prog. DN (III-IV) indication biopsies: 181 Europeans with DN
21 arrays
- Reference samples
 - Living related donor pretransplant-biopsies (LD, n=27)
 - Cadaver donor pretransplant-biopsies (CD, n=4)
 - Tumor nephrectomies (TN, n=5)
 - Thin membrane disease (TMD, n=5)
 - Minimal change disease (MCD, n=12)
 - Hypertensive Nephropathy (HN, n=20)
 - IgA-Nephropathy (IgA, n=27)
 - Lupus-Nephritis (SLE, n=32)
 - Membranous Nephropathy (MGN, n=17)
 - FSGS (FSGS, n=10)

Gene expression profiling



1. Affymetrix GeneChip

HG_U133A+B

2. Analyses



Data processing pipeline:



(Reich, *Nat. Gen.*, 2006)



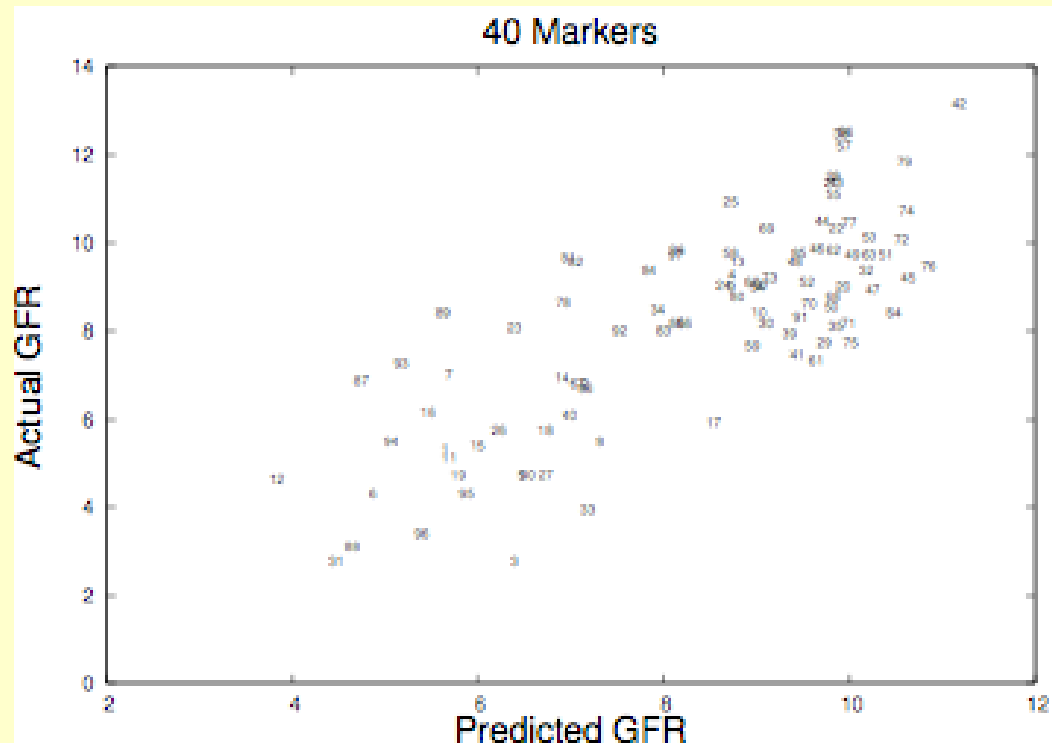
Data analysis pipeline:

NCIBI
Genomatix
Ingenuity
GeneGo

Molecular diagnostic of renal biopsies

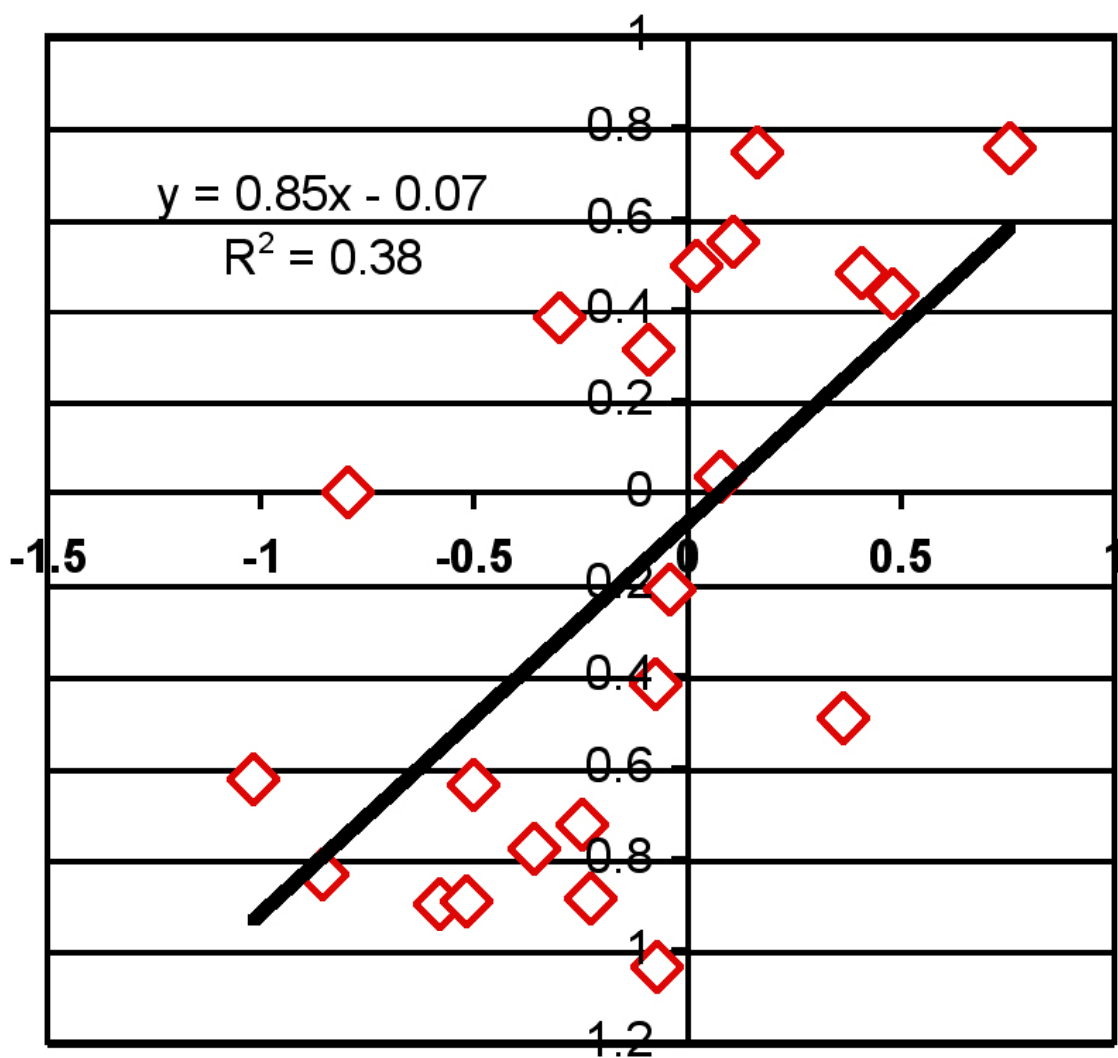
Molecular marker of progressive renal failure

- Conserved mechanism of progressive renal failure:
 - 40 markers predict decline of renal function across 5 diverse renal disease (DN,HTN, MCD,IgA,TBMD): $r=0.78$



- In independent cohort (FSGS, MGN, SLE): $r=0.69$

Prediction of renal disease progression (slope of GFR)



Predicted versus actual slope of GFR

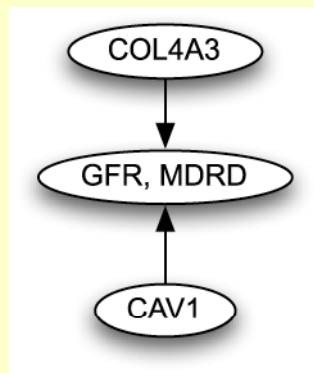
(Δ in ml/min/month):

- Independent cohort of DN: (NIDDK Pima Indian study):
- 22 patients studied with genome wide profiles
- Follow-up (Iothalamate-GFR): 7.6 years
- Ridge regression analysis top 40 CKD predictor
- 18 of 22 DN patients correctly classified ($R^2=0.38$, $p<0.05$)

Molecular definition of renal disease

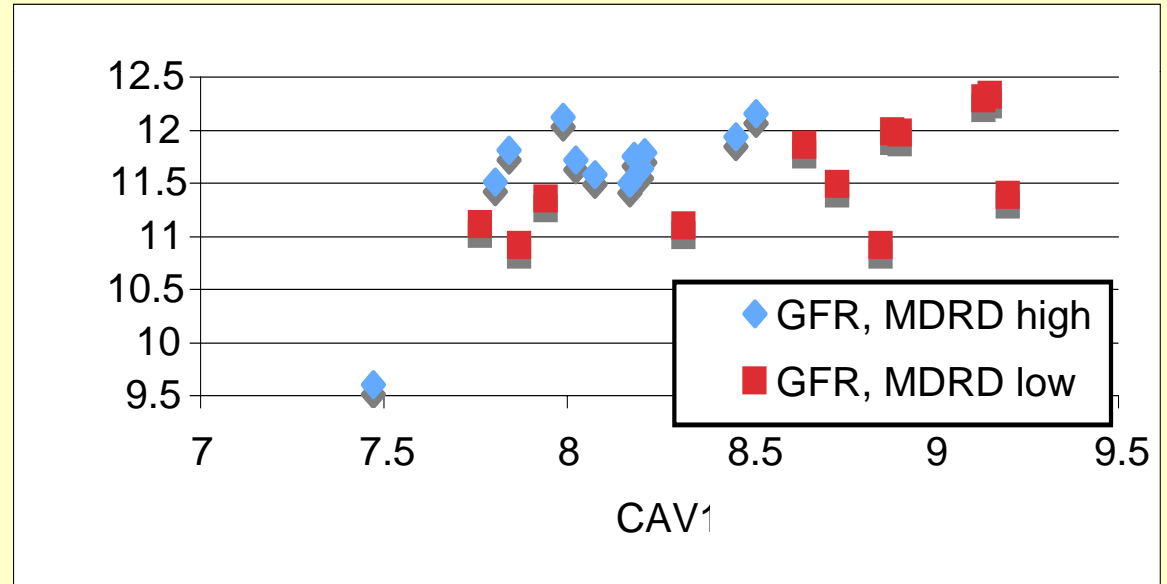
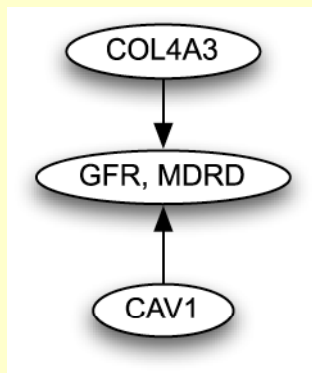
Bayesian prediction of GFR in DN

- Predicting clinical outcome in diabetic nephropathy:
Bayesian network (P.Woolf):
 - Input (Parent)
 - RNA fingerprints in early human diabetic glomerulopathy
 - Output (Child)
 - Impairment of renal function
 - (Glomerular filtration rate, (GFR), MDRD formula)



Molecular marker of diabetic end-organ damage

- Predicting clinical outcome in diabetic nephropathy: Bayesian network (P.Woolf):
 - Input (Parent)
 - RNA fingerprints in early human diabetic glomerulopathy
 - Output (Child)
 - Impairment of renal function
 - (Glomerular filtration rate, (GFR), MDRD formula)

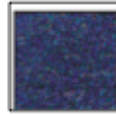


- Collagen IV alpha 3 and Caveolin-1 relationship to GFR:
 - Key molecules for intact filter in animal models
 - Candidate predictors for non-invasive screening

Integrative systems approach to renal disease

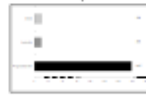
- Harness comprehensive data sets of gene expression profiling in human diabetic disease:
- Integration of
 - Gene expression profiling
 - Gene ontology classification
 - Pathway mapping
 - Promoter modelling
 - Gene expression prediction and verification
- Inflammatory response in progressive diabetic nephropathy for proof of concept study

Genome wide expression profile:
Disease specific expression
signature



*Differentially regulated
mRNAs in DN vs. control*

Defining functional categories of
expression signature
in Gene ontology (GO)



*Ontology categories in
progressive DN:
Inflammation-stress response*

Selection of relevant pathway
(prior knowledge or unbiased)
from GO category of interest



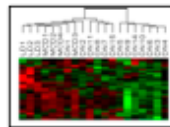
NF-κB pathway mapping in DN

Prediction of transcriptional
consequences
by activated pathway

Public database
Search tools

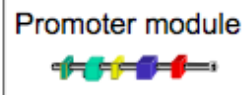
*NF-κB dependent molecules
retrieved from public database*

Evaluation of predicted mRNA
levels in expression signature



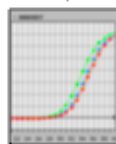
*Progressive DN shows
sig. regulation of NF-κB
dependent molecules,
sufficient to define
progressive DN*

Definition of shared
promoter modules in pathway
regulated mRNAs



*Screening of NF-κB associated
modules,
identification of NFKB_IRF1*

Analysis of promoter module
predicted mRNAs



*Expression analysis
confirming 7/9 predicted mRNAs
induced in progressive DN*

Jak/Stat pathway in tubulo-interstitium of progressive human DN

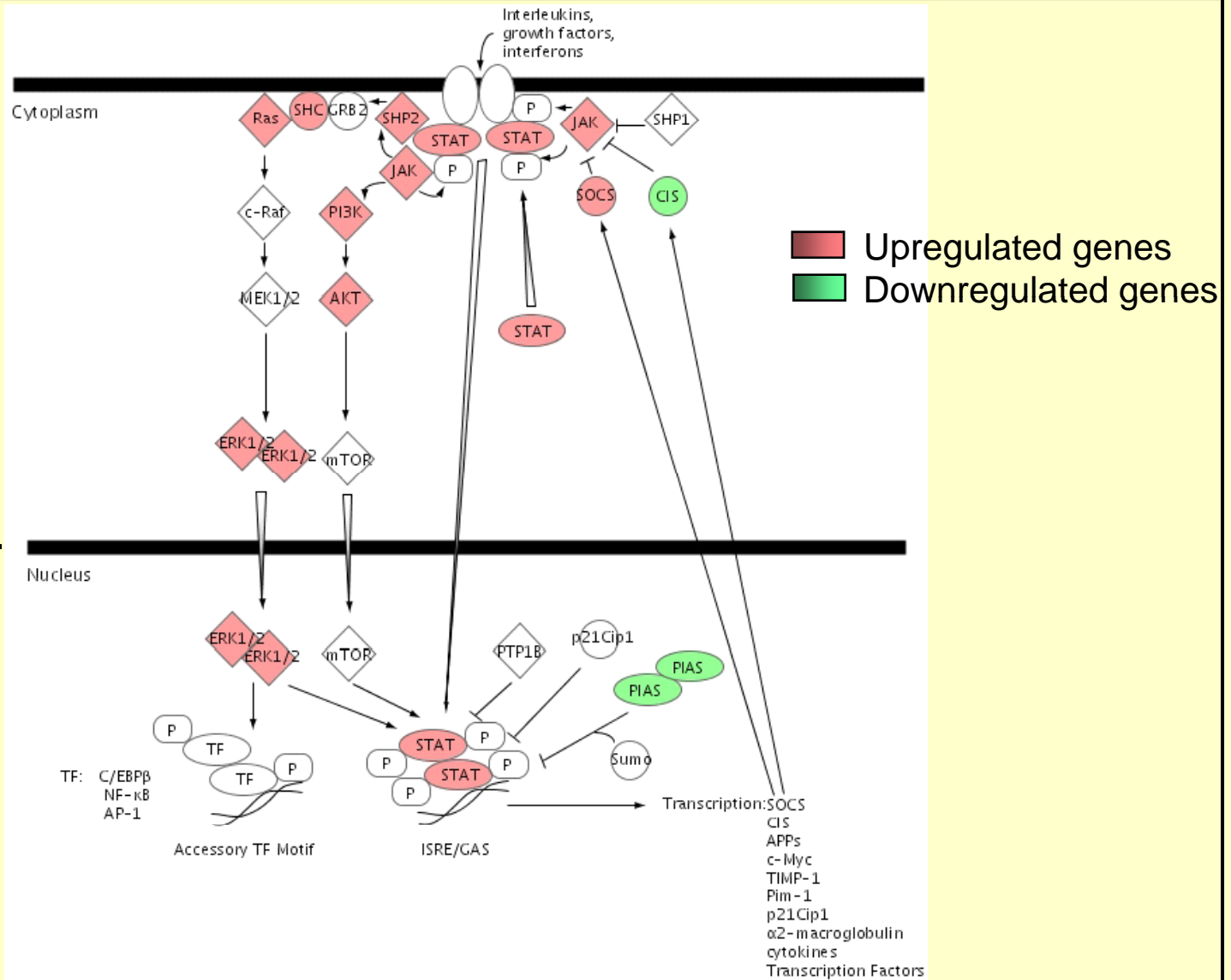


Ligands

Receptors

Cytoplasmic activation cascade

Transcriptional regulation



Jak2 mRNA expression: qRT-PCR

DN type II

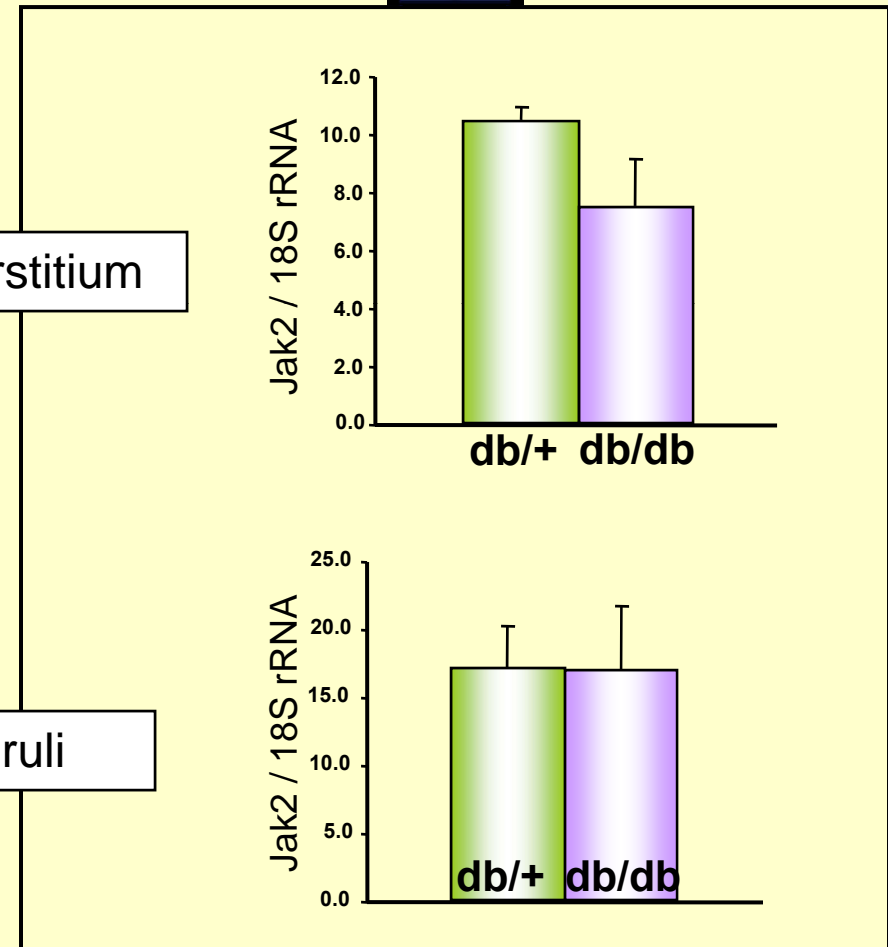
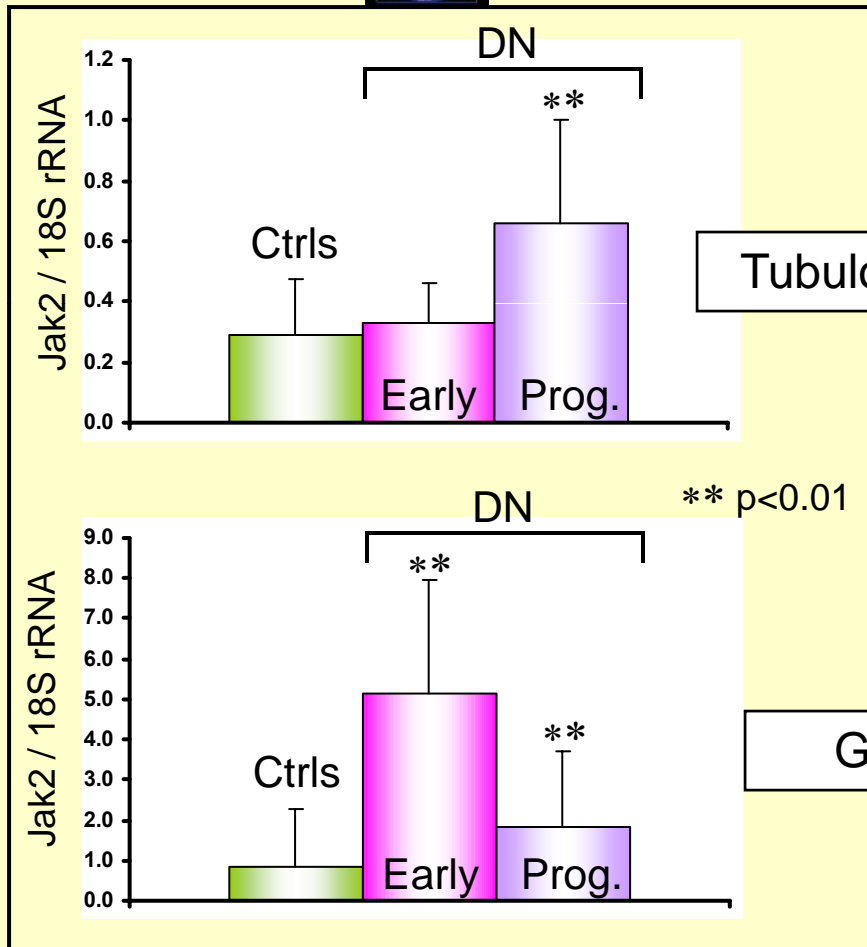


Pima Indians,
Caucasians

DN type II



C57BL/6J
db/db

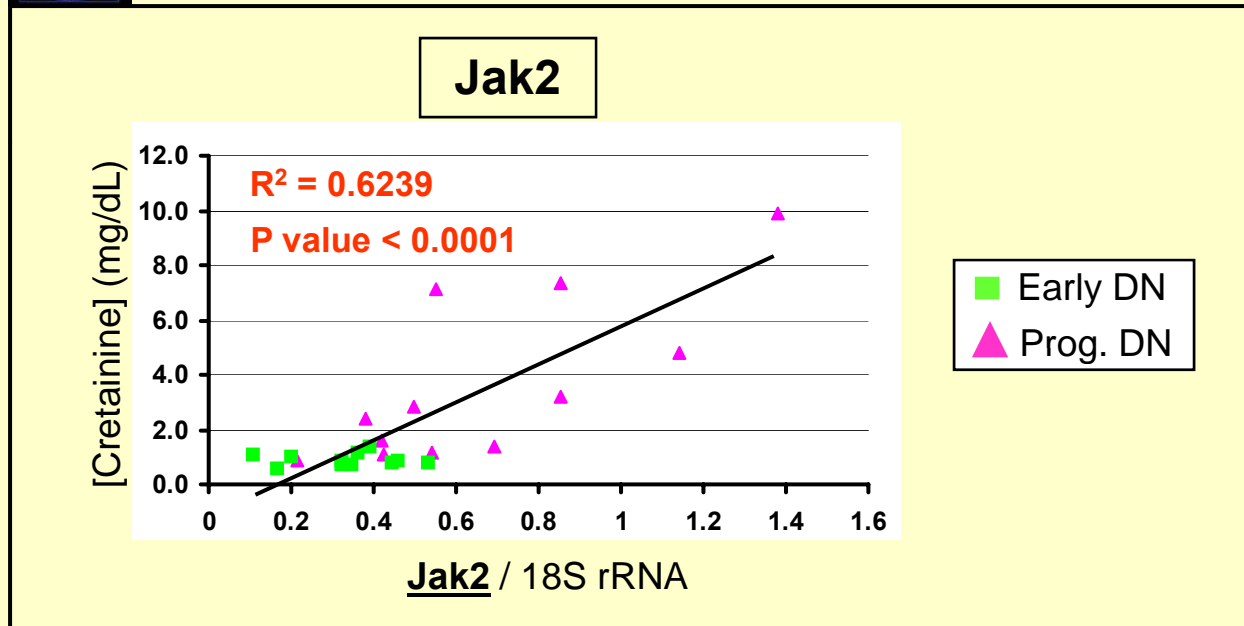


- ➔ Jak2 is significantly induced:
- In tub. of prog. DN (European diabetics) than in Ctrl.
 - In glom. of early DN (Pima protocol biopsies) than in Ctrl.

Jak2 mRNA expression in tubulo-interstitium: correlation with renal function



Pima Indians,
Caucasians



- ➔ Tubulo-interstitial Jak2 mRNA expression strongly correlates with the serum creatinine levels of the human type 2 DN patients. This also true for Jak1, Jak3, Stat1 and Stat3.
- ➔ These data strongly suggest a direct relationship between Jak/Stat levels and progression of renal insufficiency in DN.

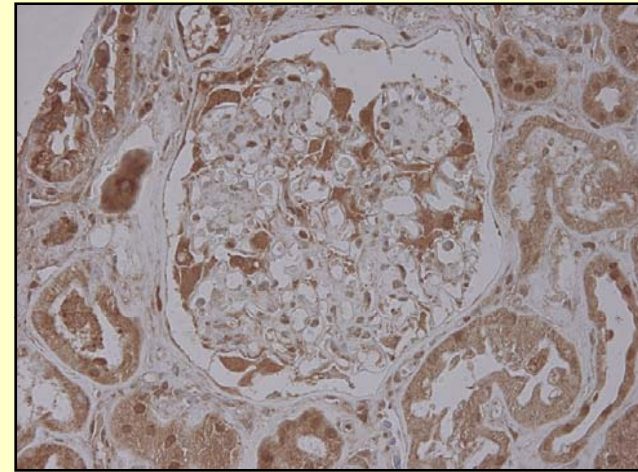
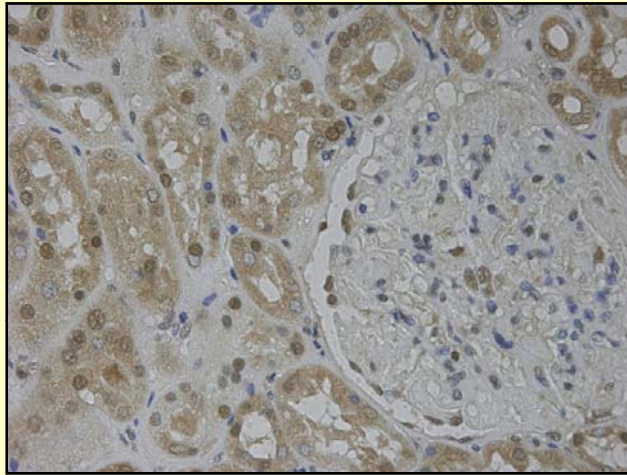


Jak staining in human kidney

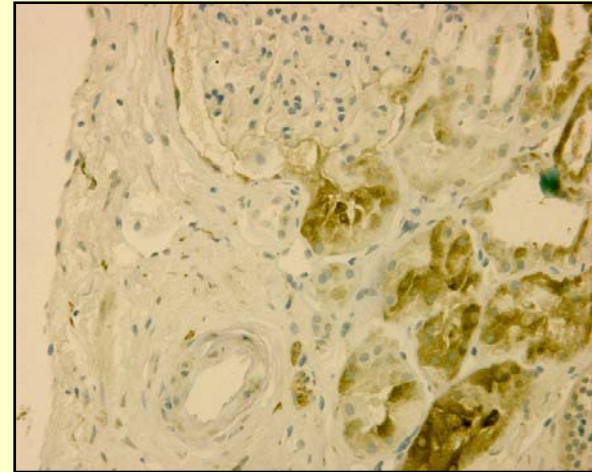
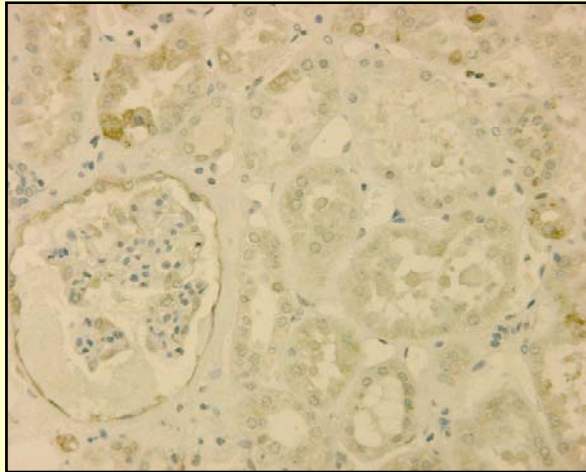
Normal

Diabetic

Jak2



Jak3

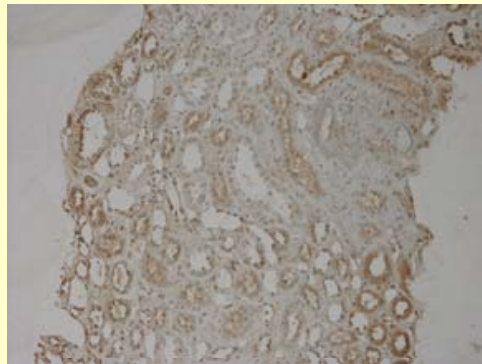


➔ Increased expression of Jak2 and Jak3 in DN compared to normal kidney.

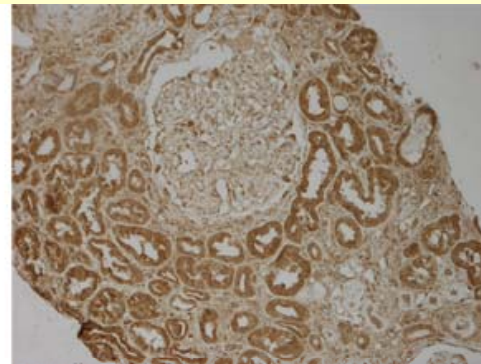
Jak2 in Human Progressive DN and non-diabetic Kidney Diseases



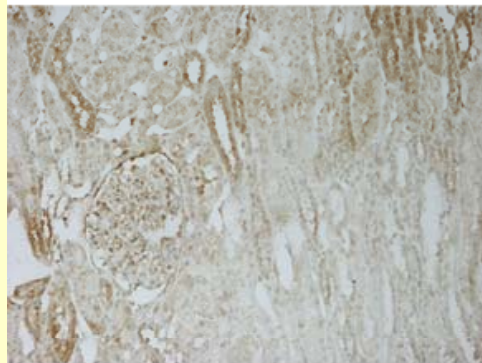
Jak2



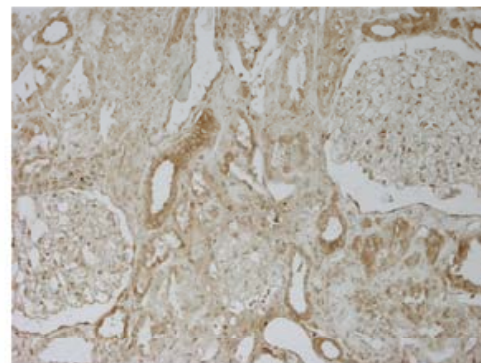
CTRL



DM

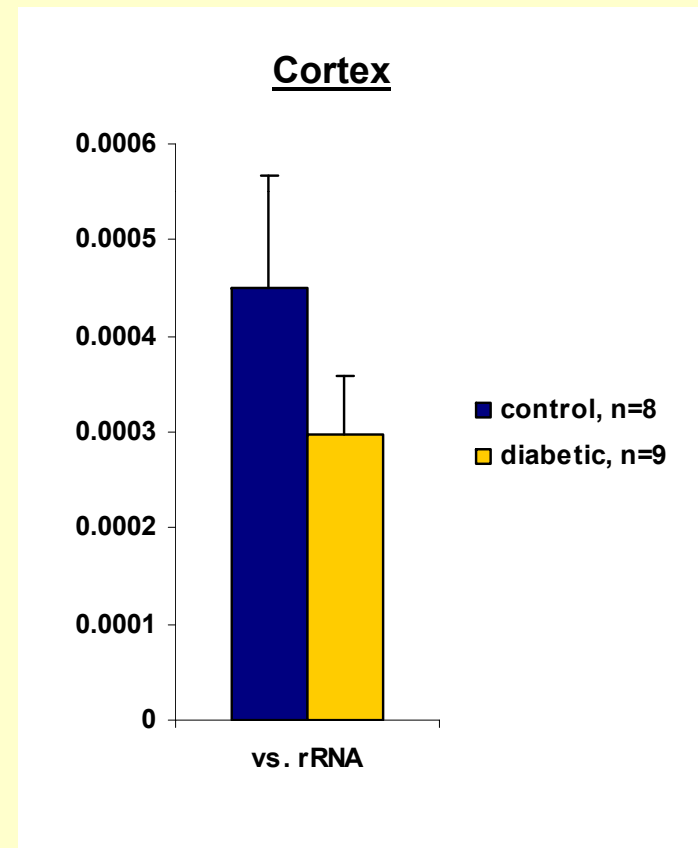
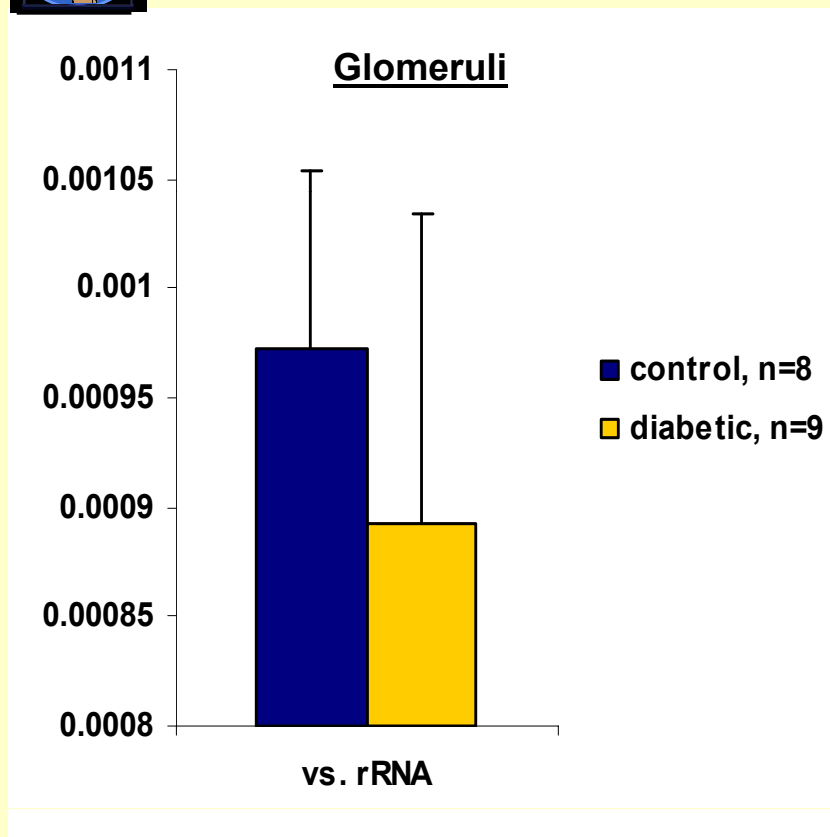


SLE



HTN

mJAK2 mRNA expression in STZ DBA Mouse (12 wk of diabetes)

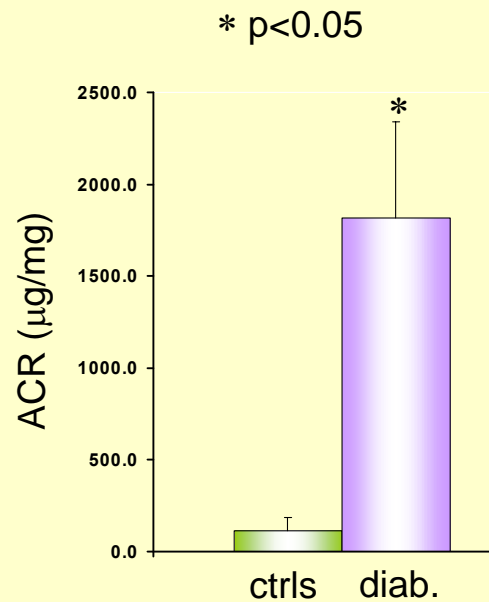


t-Test, $p > 0.05$

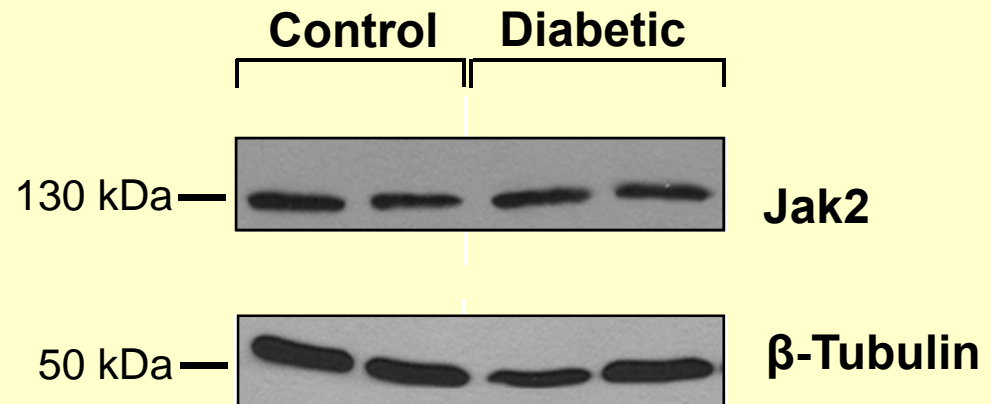
JAK2 protein expression in Glomerular lysates STZ DBA/2J Mouse (12 wk of diabetes)



Albuminuria



Western Blot Jak2

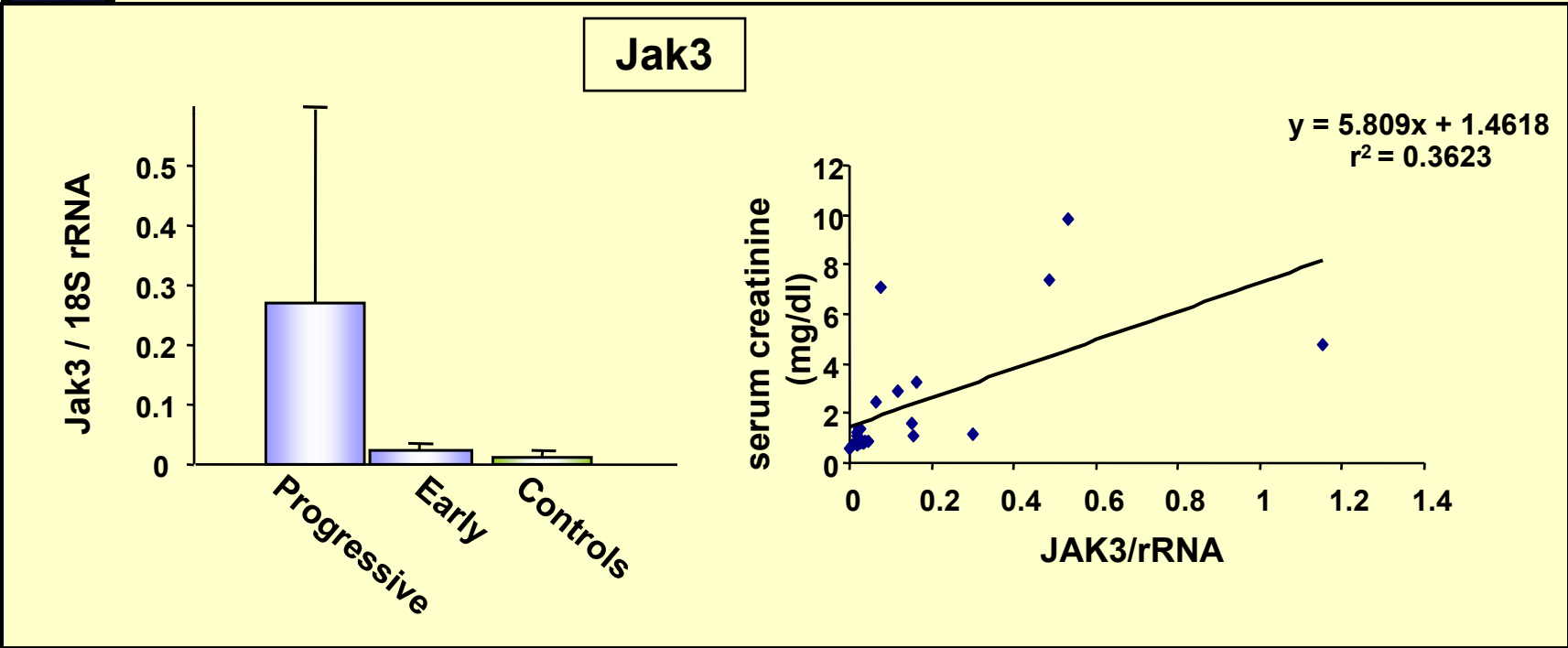


➔ Jak2 is not induced in glomeruli of DBA/2J mice with the most robust induction of albuminuria.

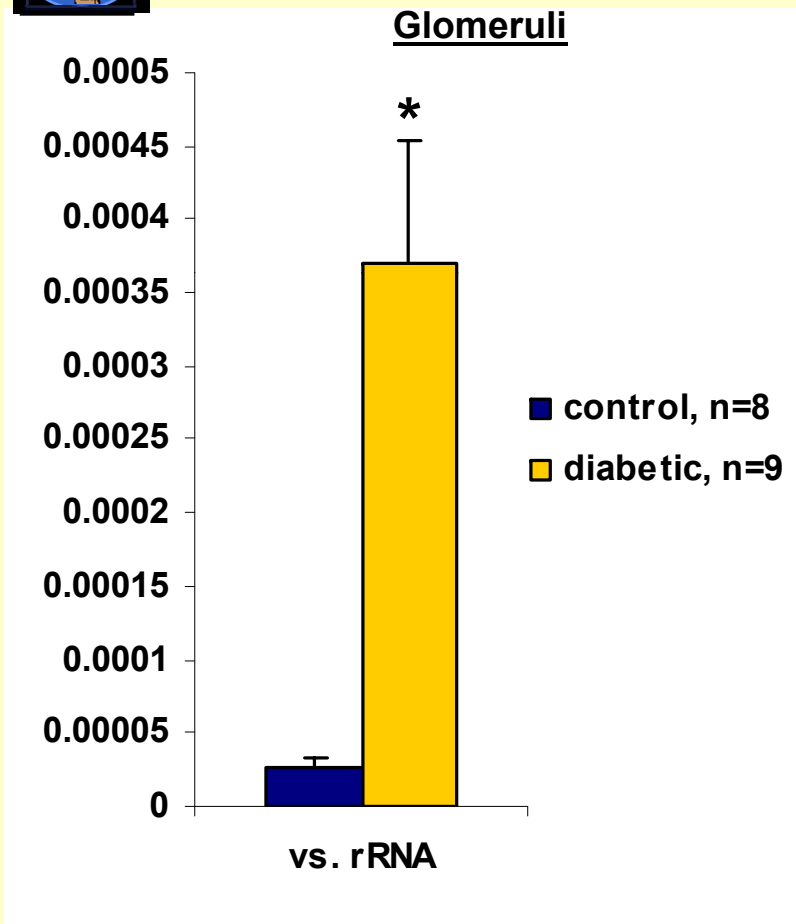
JAK3 mRNA expression in tubulointerstitium: correlation with renal function



Pima Indians,
Caucasians

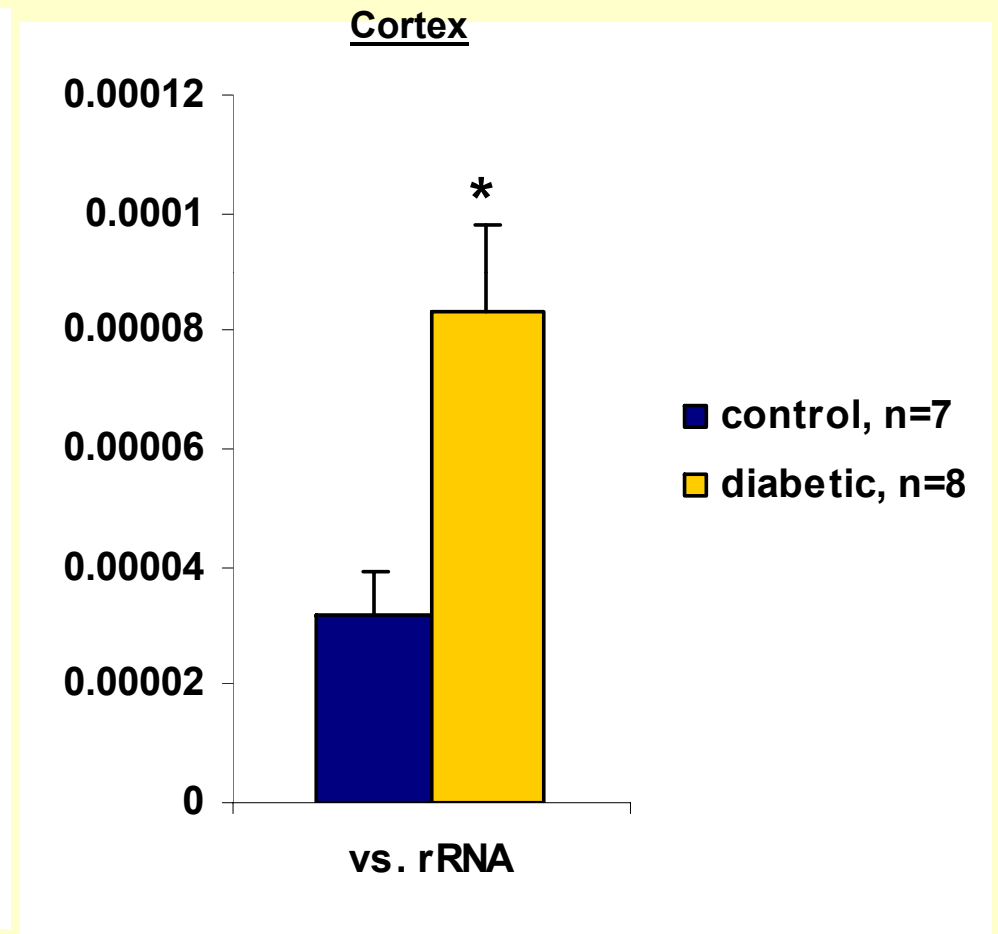


JAK3 mRNA expression in STZ DBA/2J Mouse (12 wk of diabetes)



t-Test, $p=0.0014$

* vs. control



t-Test, $p=0.0119$

* vs. control

JAK/STAT Pathways in Human Diabetic Nephropathy

Invited Review

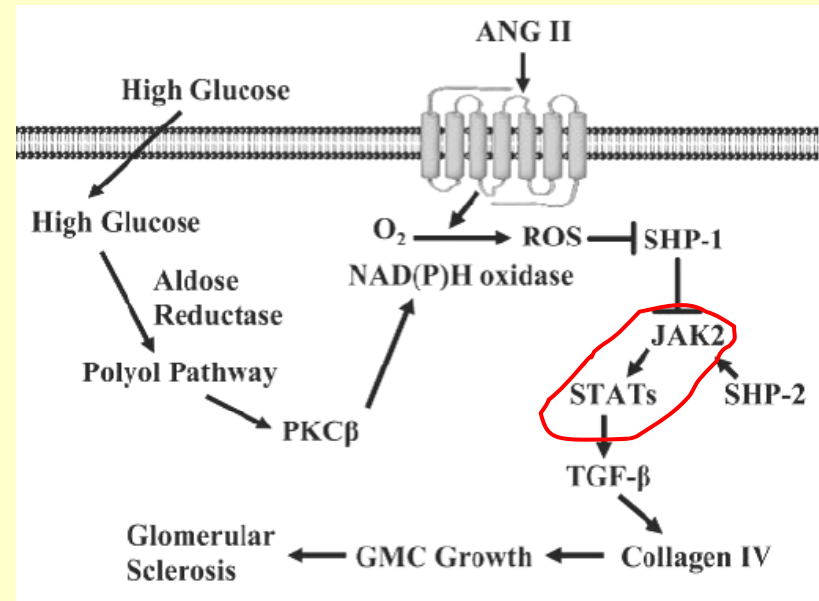
Am J Physiol Renal Physiol 290: F762–F768, 2006; doi:10.1152/ajprenal.00181.2005.

Role of the JAK/STAT signaling pathway in diabetic nephropathy

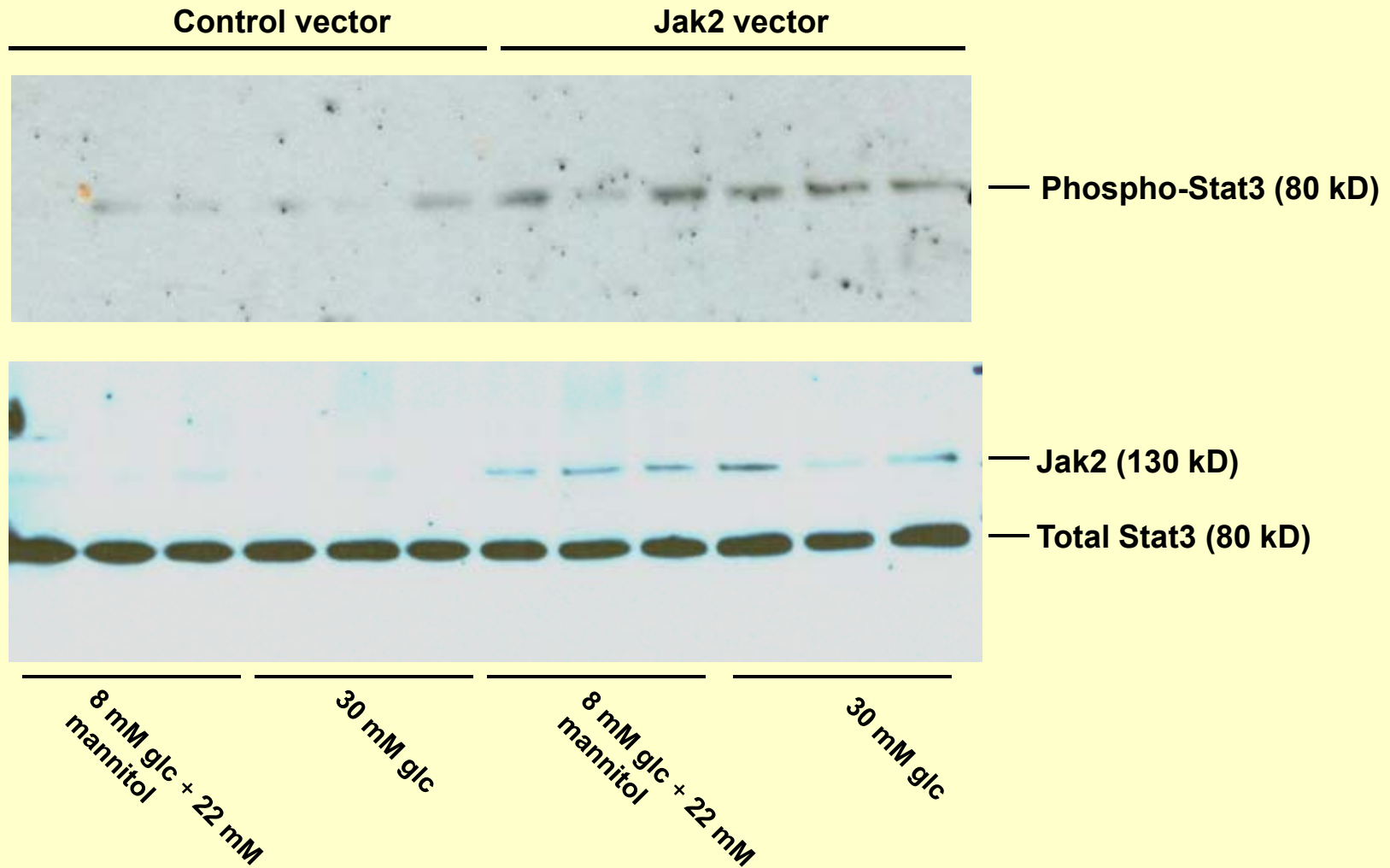
Mario B. Marrero,¹ Amy K. Banes-Berceli,¹ David M. Stern,² and Douglas C. Eaton³

¹Vascular Biology Center and ²Department of Medicine, Medical College of Georgia, Augusta; and ³Center for Cell and Molecular Signaling and Department of Physiology, Emory University School of Medicine, Atlanta, Georgia

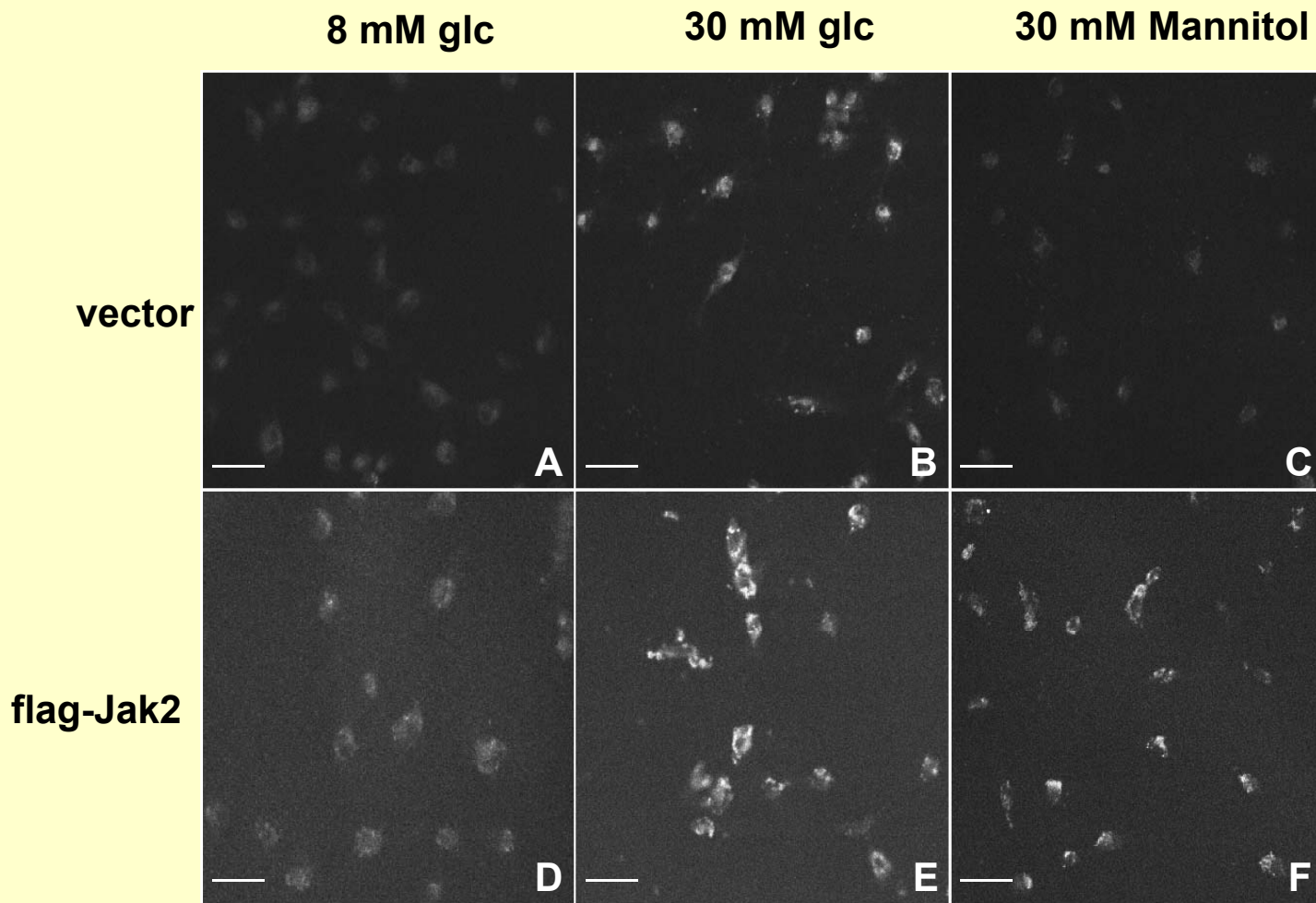
Marrero, Mario B., Amy K. Banes-Berceli, David M. Stern, and Douglas C. Eaton. Role of the JAK/STAT signaling pathway in diabetic nephropathy. *Am J Physiol Renal Physiol* 290: F762–F768, 2006; doi:10.1152/ajprenal.00181.2005.— Excessive cellular growth is a major contributor to pathological changes associated with diabetic nephropathy. In particular, high glucose-induced growth of glomerular mesangial cells is a characteristic feature of diabetes-induced renal complications. Glomerular mesangial cells respond to traditional growth factors, although in diabetes this occurs in the context of an environment enriched in both circulating vasoactive mediators and high glucose. For example, the vasoactive peptide ANG II has been implicated in the pathogenesis of diabetic renal disease, and recent findings suggest that high glucose and ANG II activate intracellular signaling processes, including the polyol pathway and generation of reactive oxygen species. These pathways activate the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling cascades in glomerular mesangial cells. Activation of the JAK/STAT signaling cascade can stimulate excessive proliferation and growth of glomerular mesangial cells, contributing to diabetic nephropathy. This review focuses on some of the key elements in the diabetic microenvironment, especially high glucose and the accumulation of advanced glycoxidation end products and considers their impact on ANG II and other vasoactive peptide-mediated signaling events in vitro and in vivo.



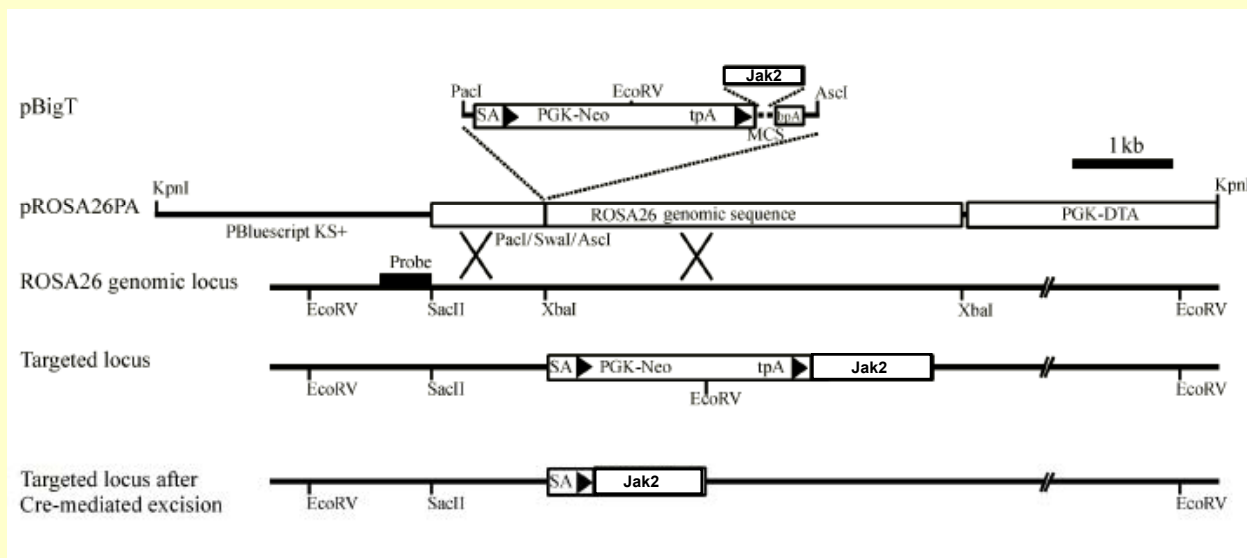
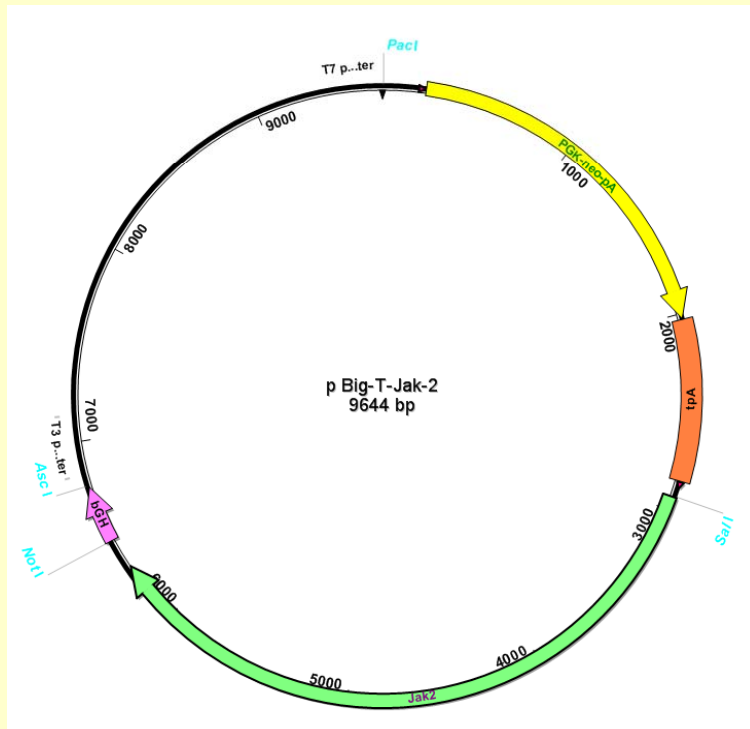
Increased Stat3 phosphorylation with Jak2 overexpression



Increased ROS (DCFDA staining) with Jak2 overexpression



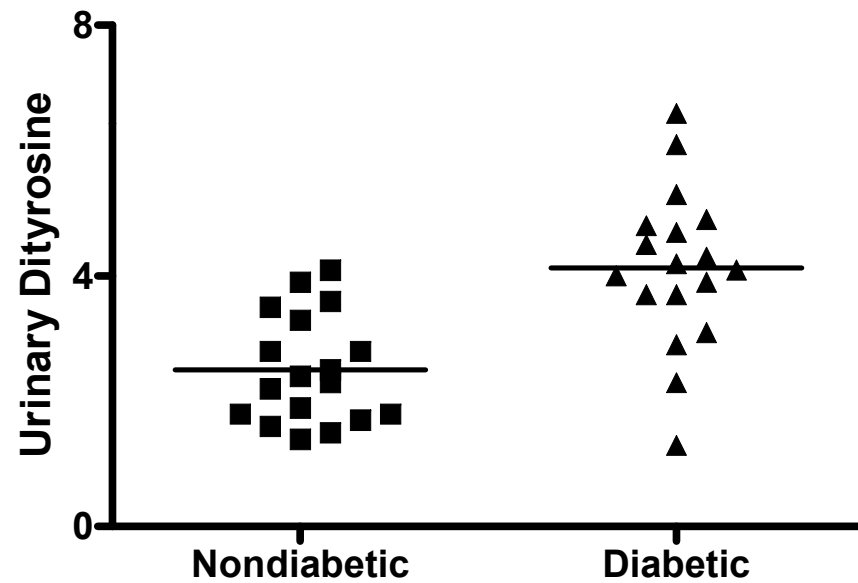
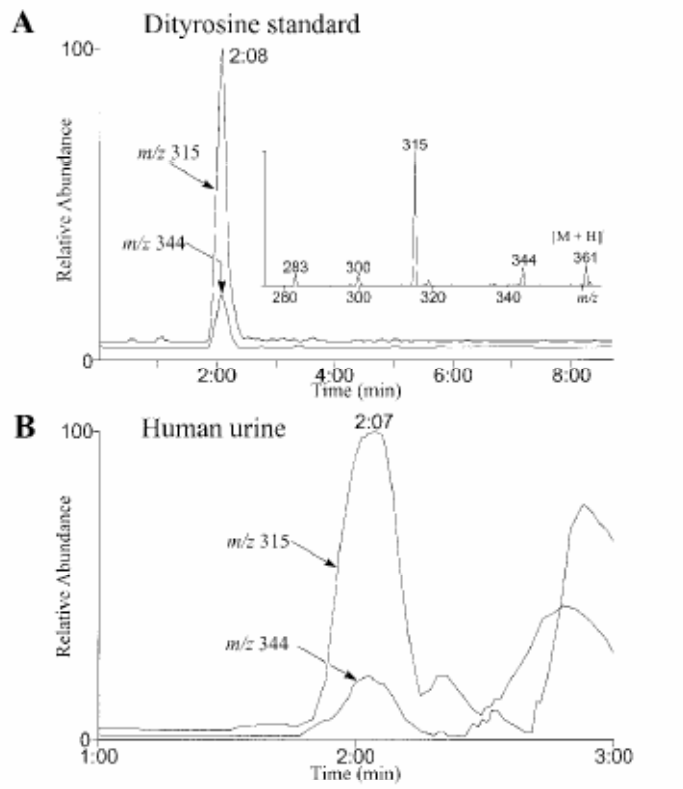
ROSA26/JAK2 Knockin Model



Future Plans:

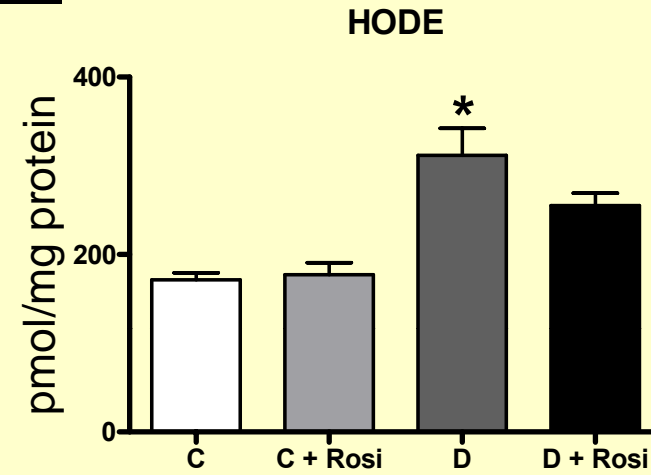
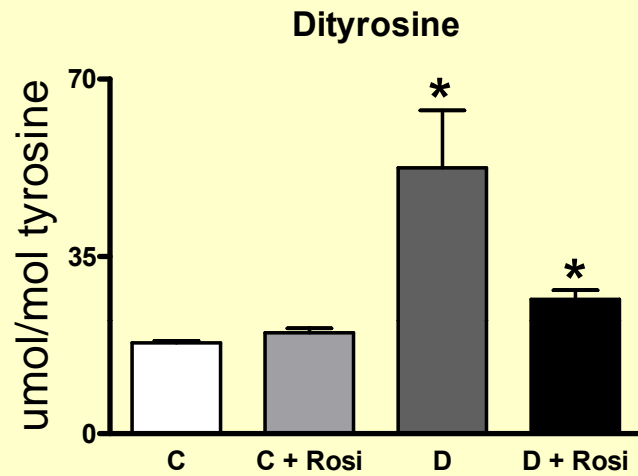
- **Generation of podocyte and proximal tubular specific Jak2 transgenic mice**
 - Nphs2-Cre mouse used successfully previously
 - generate podocyte specific Jak2 overexpression.
 - Proximal tubule specific Cre mouse?
 - Proximal tubule specific Jak2 overexpressing mice.
- **Mechanistic studies to examine effects of Jak2 and Jak3 overexpression**
- **Gene expression studies (M.Kretzler):**
 - Jak 2 dependant pathways and transcriptome
- **Metabolome studies (S.Pennathur):**
 - Modulation of oxidative stress response

Urinary Dityrosine level is elevated in diabetic patients with microvascular disease

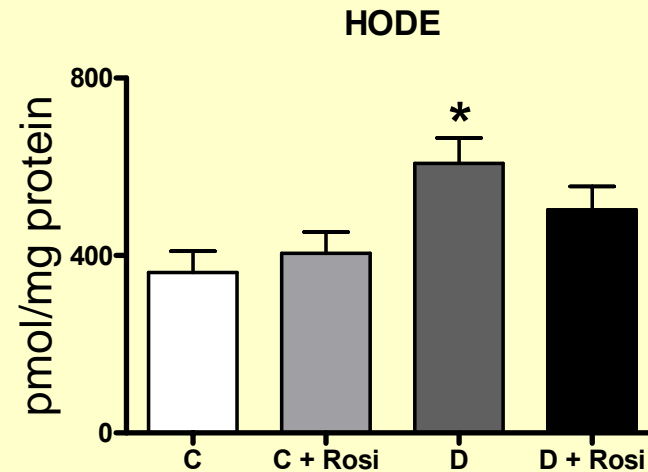
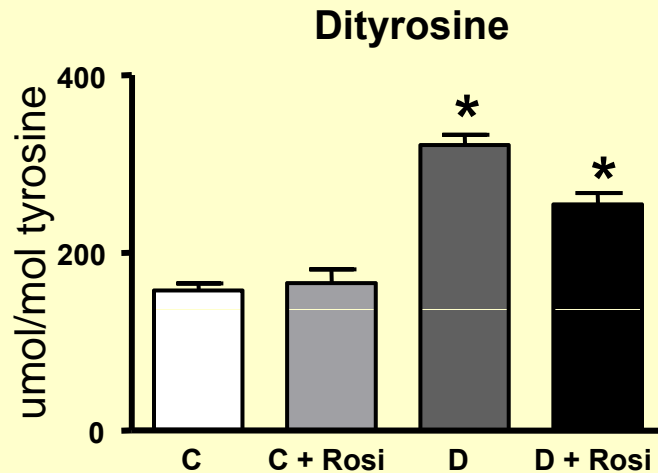


TZD reduction of oxidant products in STZ DBA/2J mice

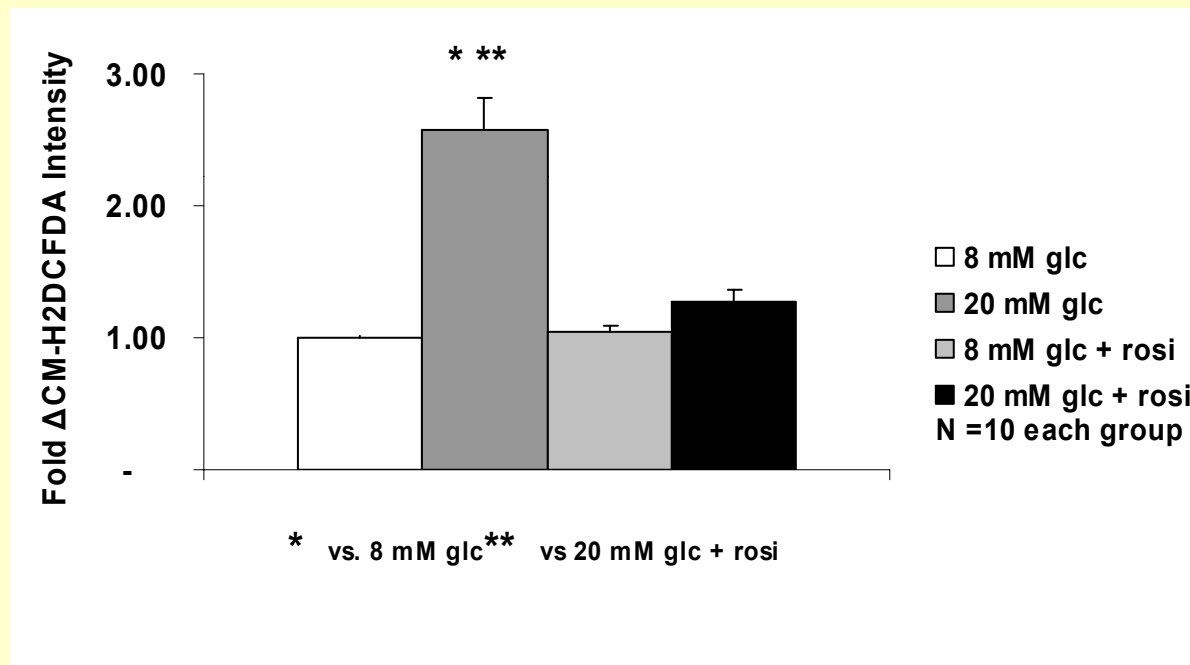
Plasma



Kidney



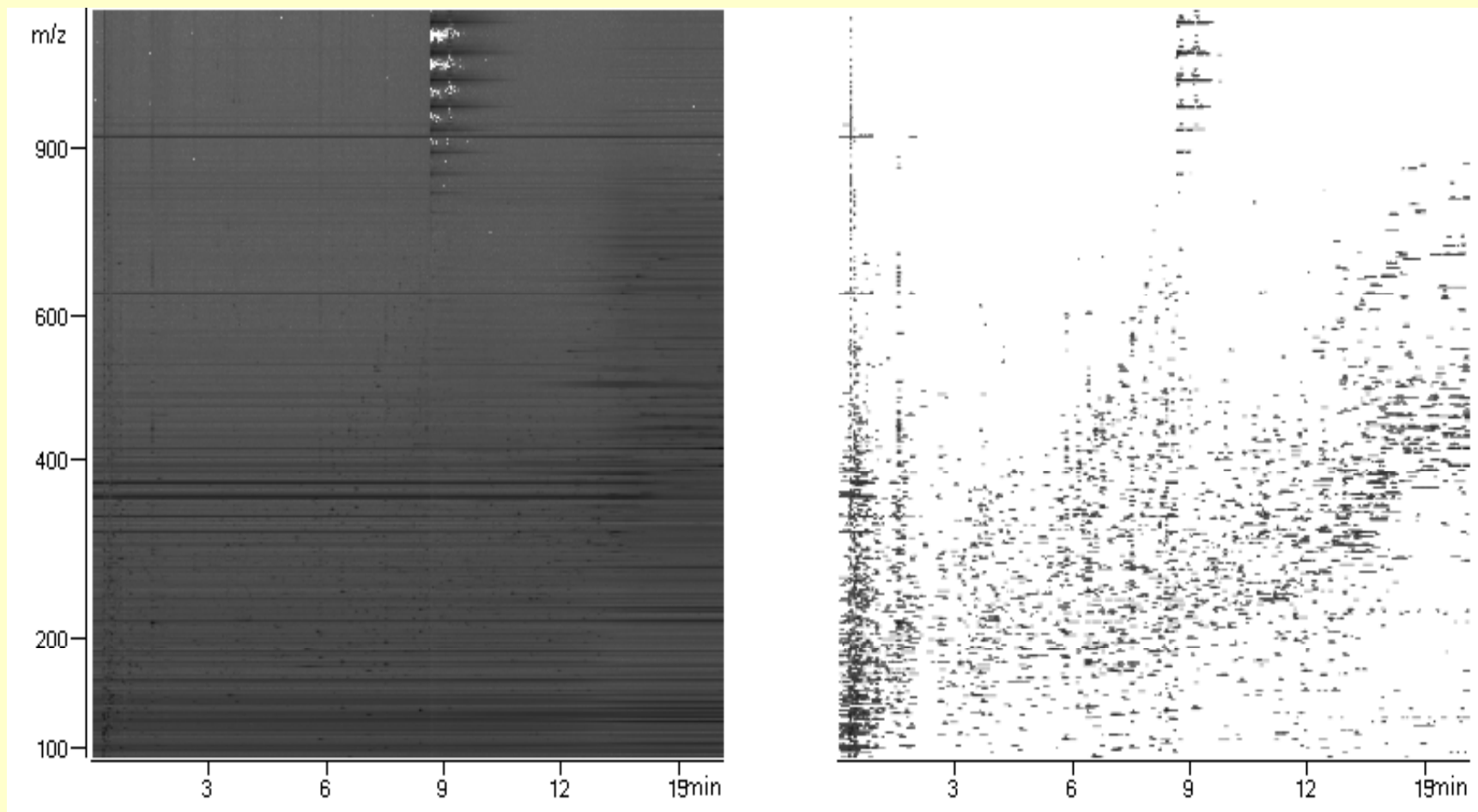
TZD reduction of ROS accumulation in cultured rat mesangial cells



Metabolite Plot of Control DBA/2J Urine Sample before and after deconvolution.

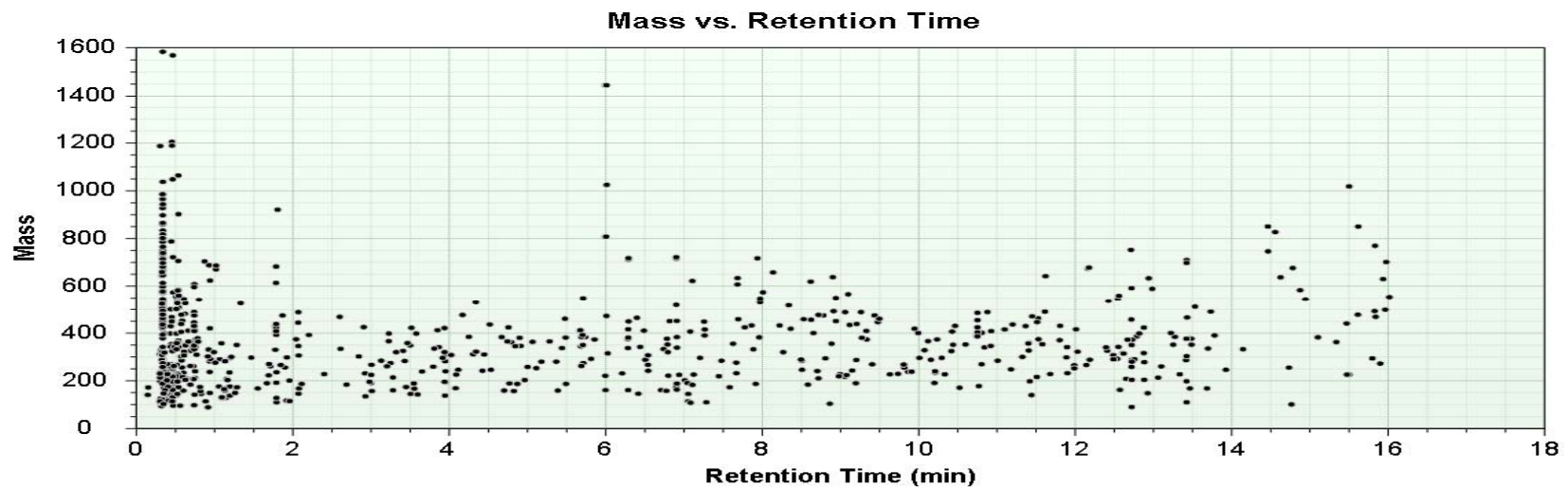
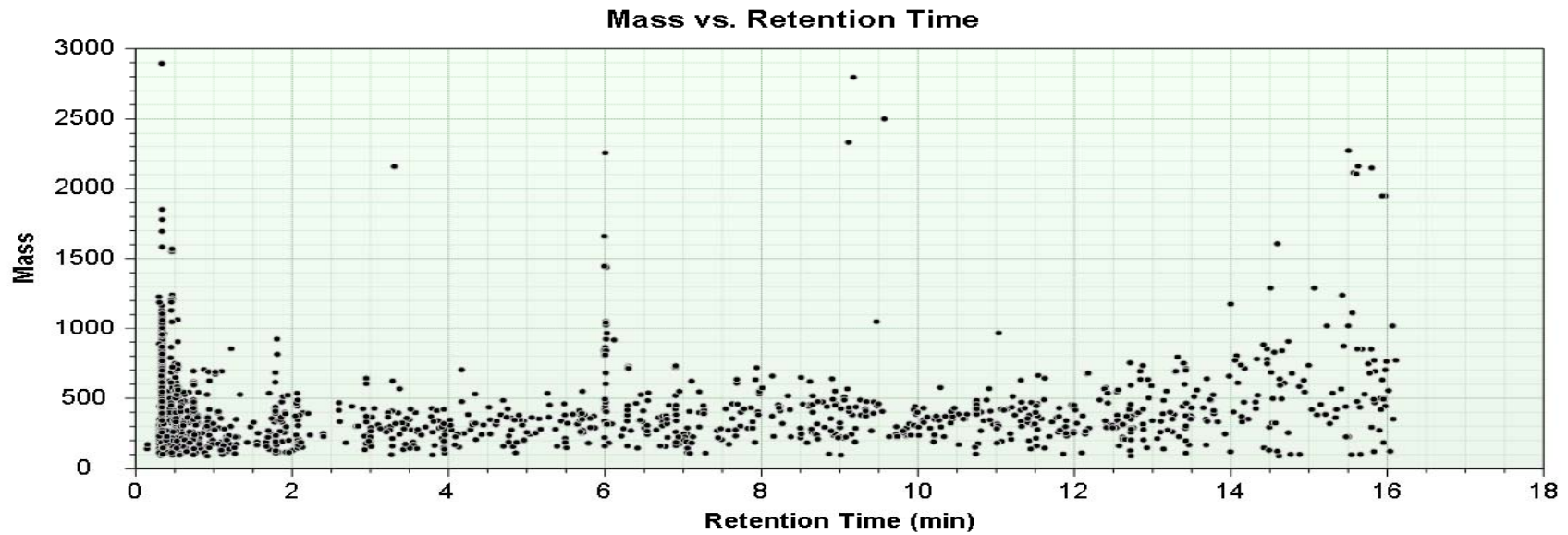
Before

After



3602 Features were identified by ESI(+) Control Urine Sample

Electrospray Ionization (Positive mode) of urine from diabetic DBA/2J mice +/- rosiglitazone



Metabolite Identification M=113 Creatinine

Export 3614 features

Featur	RT	mass	mass SD	abund.	satur.	height
1	1	16.084	412.2595		2061367	285655.1875
2	2	0.451	131.0693		1193994	878595.5625
3	3	0.542	143.0944		1158239	676490.0625
4	4	0.366	113.0592		1121829	982256.8125
5	5	1.553	192.0273	0.0016	1089506	318809.8125
6	6	0.739	97.9770	0.0005	911729	191288.15625
7	7	0.438	125.0143		872203	548250.125
8	8	5.847	104.0276		782760	202741.53125
9	9	0.304	109.0015		685606	116021.28125
10	10	13.380	288.2668	0.0000	628323	513.12890625
11	11	0.601	351.3322		627570	51511.578125
12	12	7.522	173.1043	0.0003	583109	70400.453125
13	13	8.403	130.0416		577702	229919.875
14	14	8.719			542290	3865.7890625
15	15	15.709	412.2594		537597	39740.359375
16	16	3.531	143.0767		530117	1625.5859375
17	17	13.327	332.2934	0.0005	516322	391.57421875
18	18	8.719			503751	366.23046875
19	19	16.003	390.2770		400854	3220.0059375
20	20	15.208	418.3093	0.0005	458407	74.943359375

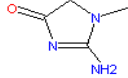
Export Feature #4 (RT=0.366)

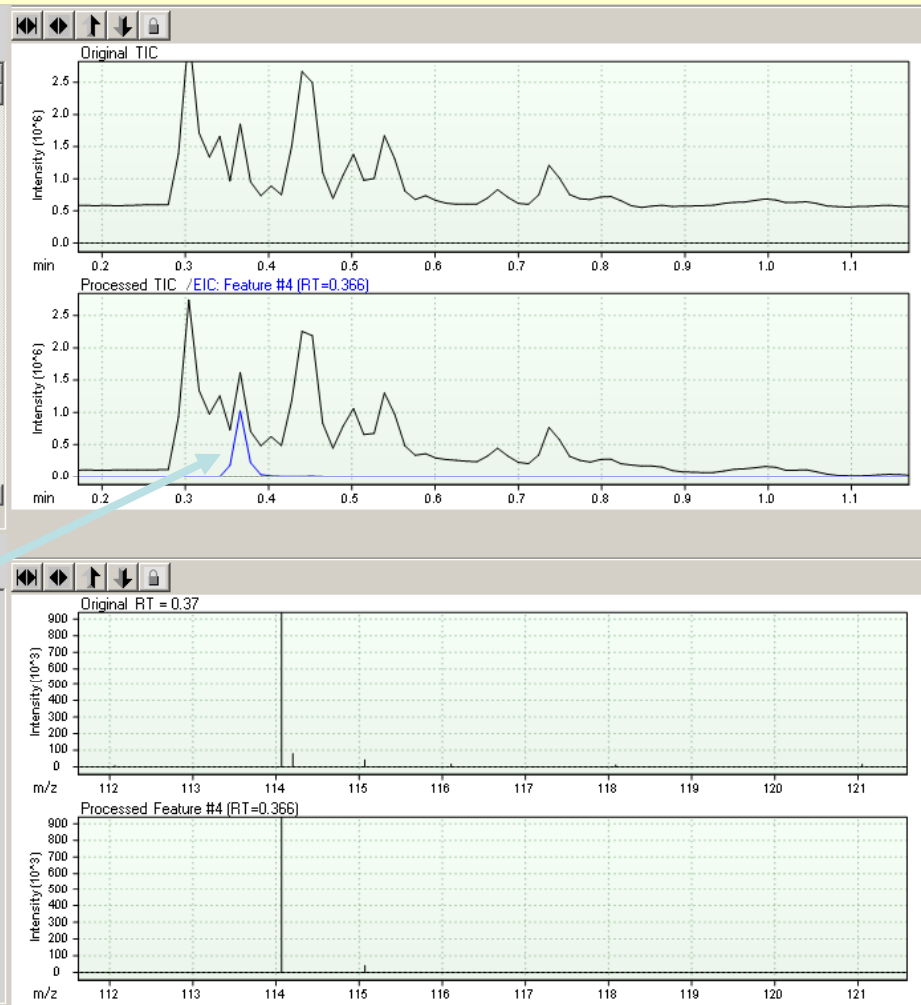
species	RT	m/z	mass	abund.	width	satur.
1	M	0.366	113.0592	1121829	0.018	
2	M+H	0.366	114.0664	113.0592	1075194	0.018
3	M+H+1	0.366	115.0689		46635	0.015
4						

Possible Compositions

Export Chemistry Compositions

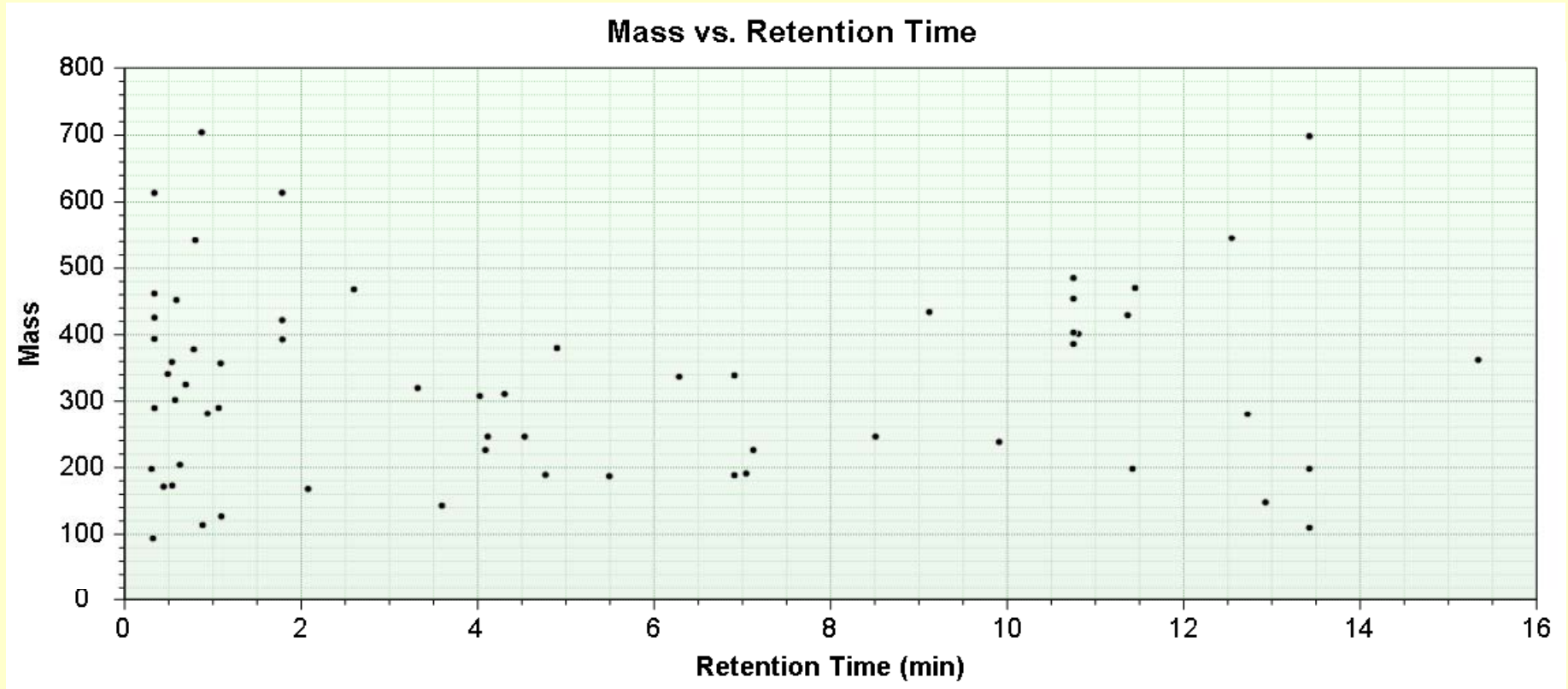
chemical formula	dm(Da)	dm(ppm)	DBE	score
1	C4H7N3O	-0.0003	-2.3	3.0 100

MID	Mass	Name	Formula	CAS	KEGG	Structure
8	113.0589	Creatinine	C ₄ H ₇ N ₃ O	60-27-5	C00791	



Metlin Metabolite Database Search within 10 ppm

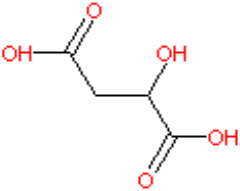
Differential Metabolite Profile: Features Found in Diabetic Urine That Return to Normal with Rosiglitazone

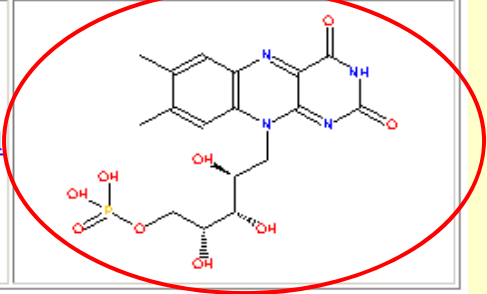


55 features found in all six ESI(+) LCMS data files with Changes of 2x or greater

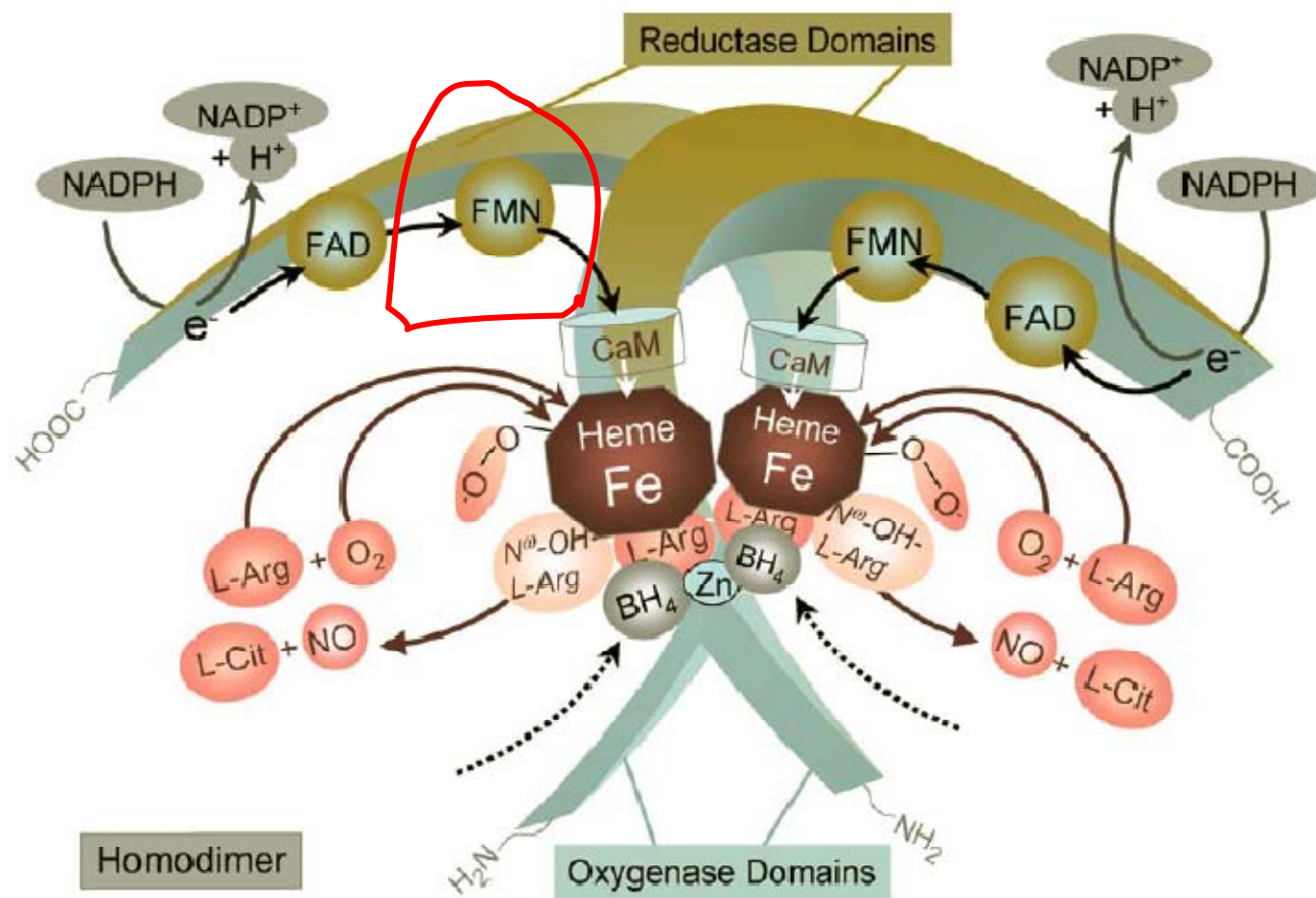
Two Features That Increased 6-Fold in Diabetic + Rosi Urine

Metlin Database Search Results Reveal that these molecular features are consistent with Malic Acid and Flavin mononucleotide.

MID	Mass	Name	Formula	CAS	KEGG	Structure
118	134.0215	malic acid	$C_4H_6O_5$	6915-15-7	C00149	

MID	Mass	Name	Formula	CAS	KEGG	Structure
2301	456.1046	Flavine mononucleotide (FMN)	$C_{17}H_{21}N_4O_9P$	146-17-8	C00061	

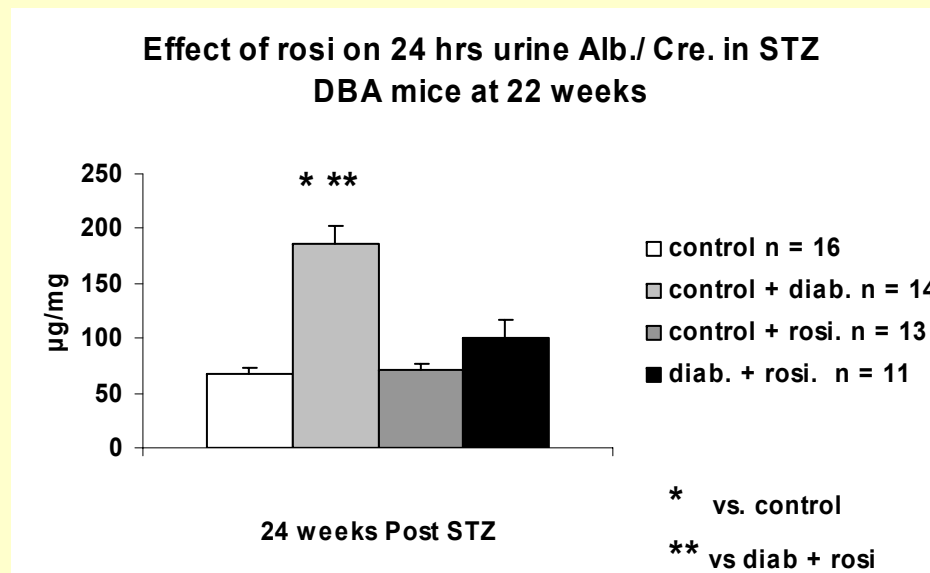
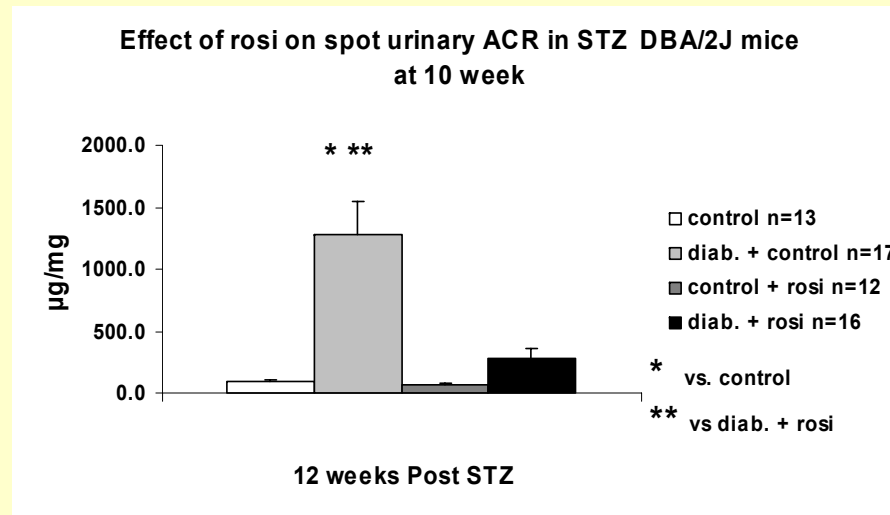
FMN is a critical co-factor in many redox enzymes



Summary

- Jak2 expression is substantially enhanced in glomerular and tubulointerstitial compartments in progressive human diabetic nephropathy but not in mouse models.
- Cortical Jak3 expression is enhanced in both humans and in mouse models.
- Tubulo-interstitial Jak2 and Jak3 expression is restricted to proximal tubule cells.
- Jak2 overexpression in mesangial cells leads to increased Stat3 phosphorylation and ROS generation.
- Due to difficulties with the SGLT2 promoter in some groups, and the advantage of an inducible model, we are generating a Jak2 knock-in to the ROSA26 locus which should allow expression in any cell type after crossing with a tissue specific Cre mouse.
- Hypothesis driven and screening study tools are in place for high resolution molecular phenotyping of the animals.

TZD Prevention of DN in STZ DBA/2J mice



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