University of Michigan Neprhopathy Update

Chip Brosius, Matthias Kretzler

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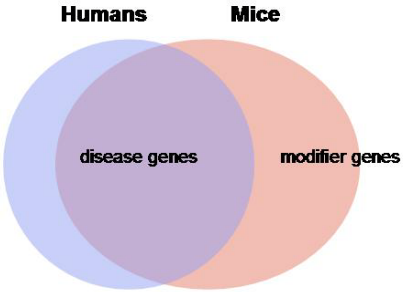
Discordance of mouse/human responses

Human



Mouse

Specific changes

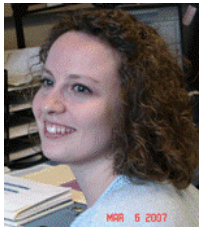
Common pathways

Specific changes

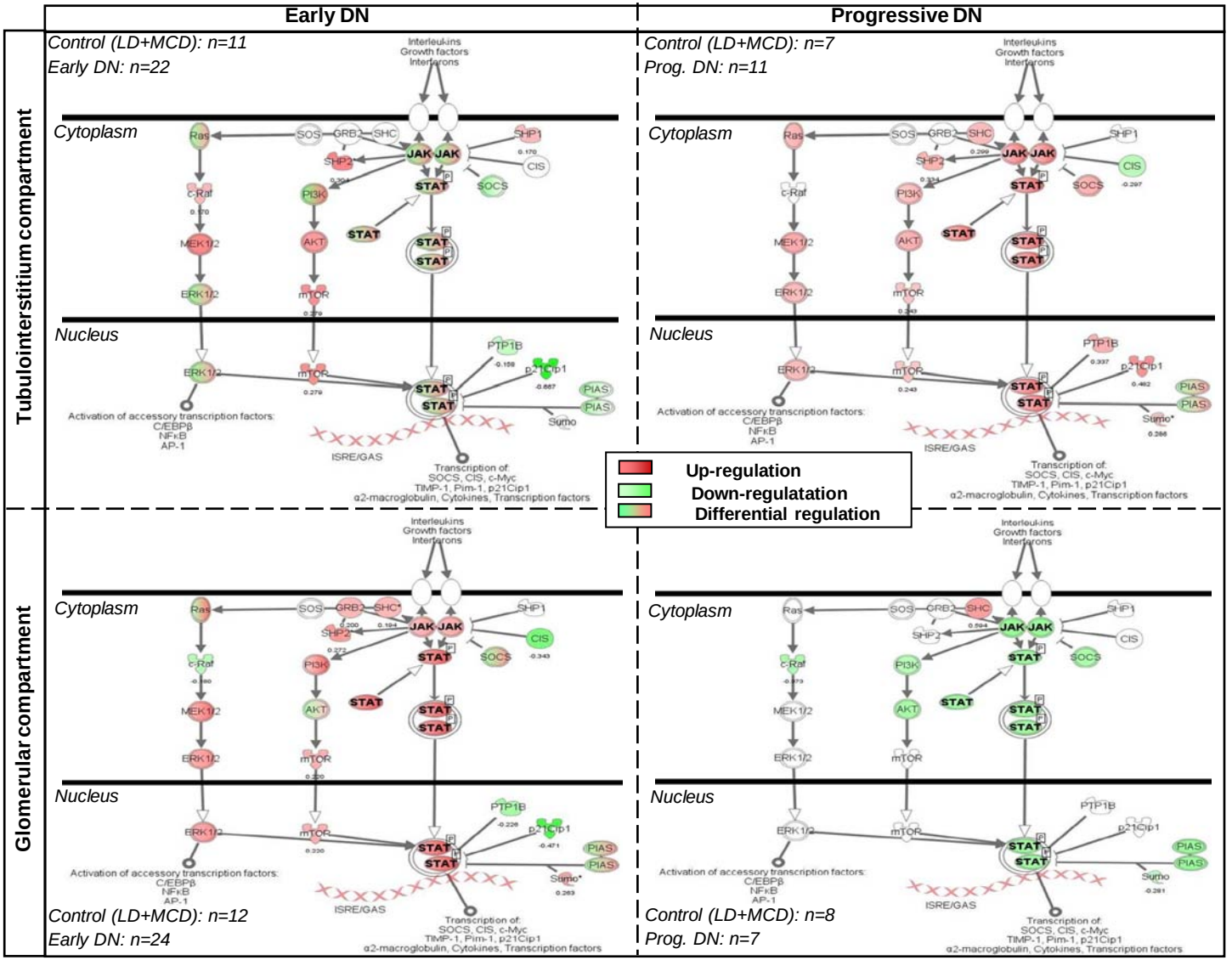
Diabetic Nephropathy

Prog. GS, tubulointerstitial fibrosis, decline in GFR

mild GS, no tubulointerstitial damage, no decline in GFR



Jak/Stat pathway in human DN



Berthier et al., Diabetes. 2009;58:469

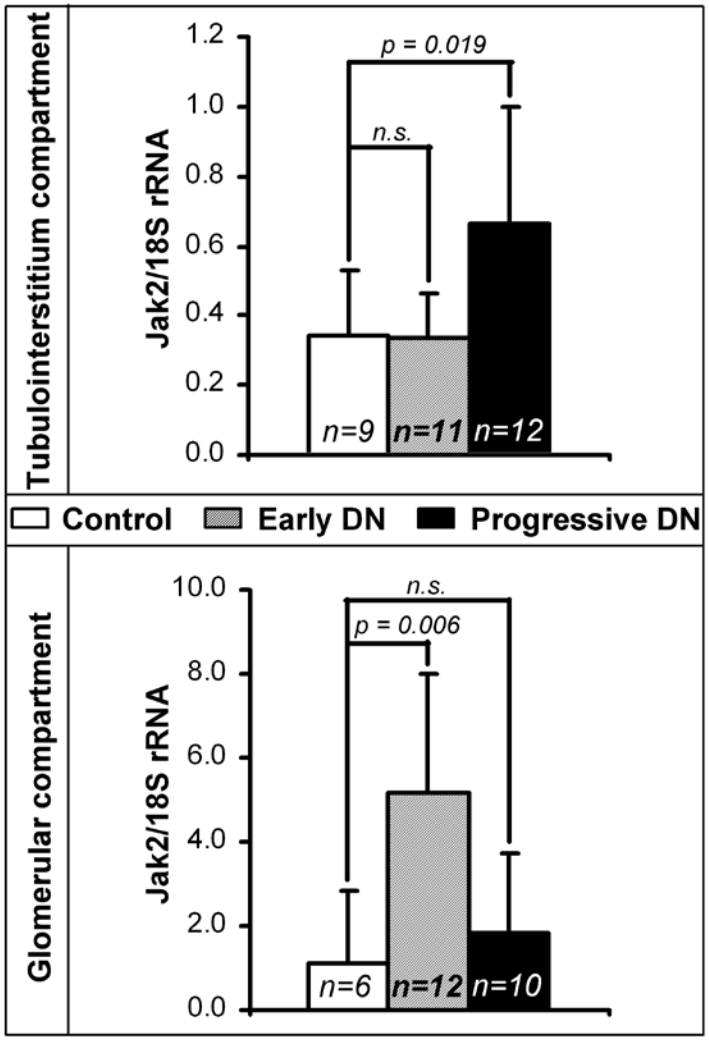
Jak/Stat pathway in human DN

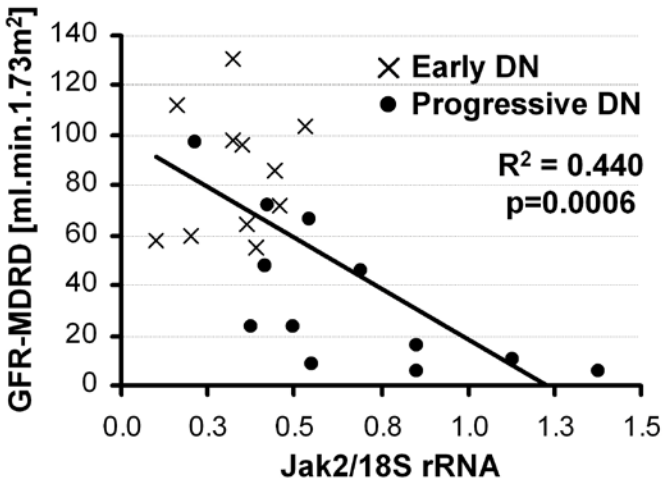
Supplementary TABLE 5A: Tubulointerstitital and glomerular Jak1, Jak2, Jak3, Stat1 and Stat3 mRNA expression values as assessed by real-time quantitative RT-PCR; data are presented as gene/18S rRNA expression ratio and as mean ± SD (p-value). A p-value<0.01 was considered to be significant compared to the controls after Bonferroni's correction (in bold). Early DN = Pima Indians, Prog. DN = European Caucasian.

	Glomerular compartment			**Tubulointerstitial compartment**		
Gene name	**Controls**	**Early DN**	**Prog. DN**	**Controls**	**Early DN**	**Prog. DN**
Jak1	0.13 ± 0.08	**0.69 ± 0.43 (0.0007)**	**0.43 ± 0.42 (0.003)**	0.81 ± 0.73	0.59 ± 0.14 (0.878)	1.29 ± 0.86 (0.023)
Jak2	0.83 ± 1.42	**5.14 ± 2.84 (0.0006)**	**1.81 ± 1.89 (0.016)**	0.29 ± 0.19	0.33 ± 0.13 (0.340)	**0.66 ± 0.34 (0.0014)**
Jak3	0.14 ± 0.39	0.44 ± 0.35 (0.032)	**0.71 ± 1.10 (0.003)**	0.01 ± 0.01	0.02 ± 0.01 (0.056)	**0.27 ± 0.32 (0.0001)**
Stat1	0.57 ± 0.67	**3.05 ± 1.61 (0.001)**	1.14 ± 1.14 (0.204)	0.26 ± 0.27	0.25 ± 0.18 (0.601)	**0.86 ± 0.80 (0.0014)**
Stat3	0.26 ± 0.17	**0.90 ± 0.37 (0.001)**	0.57 ± 0.38 (0.019)	0.74 ± 0.71	0.54 ± 0.18 (0.644)	0.71 ± 0.18 (0.175)

Berthier et al., Diabetes. 2009;58:469

Jak2 mRNA expression: qRT-PCR

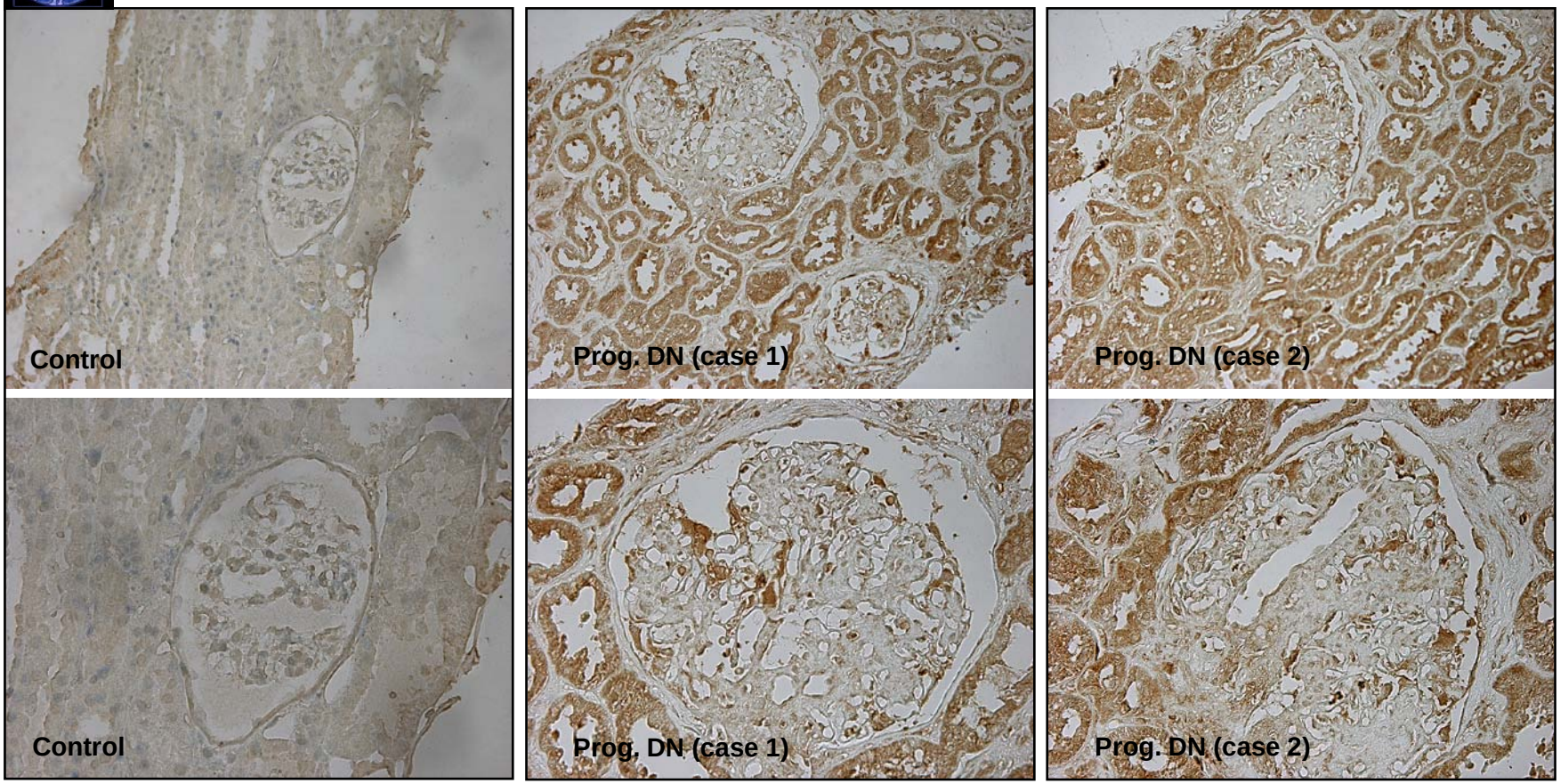




Tubuloint. Jak2 mRNA expression inversely correlates with eGFR.
This also true for Jak1, Jak3, Stat1 and Stat3.

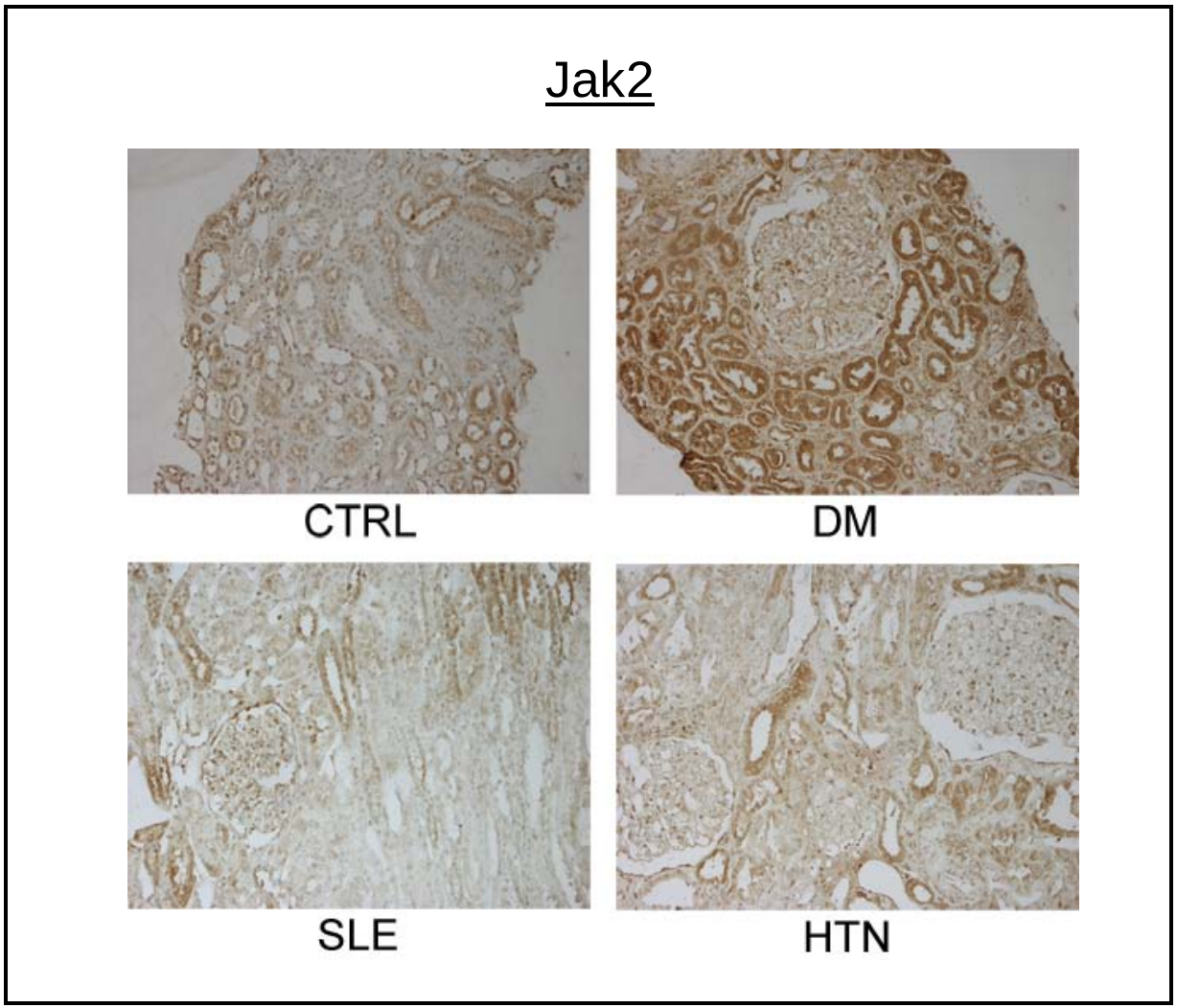
Berthier et al., Diabetes. 2009;58:469

Jak2 immunostaining in human kidney



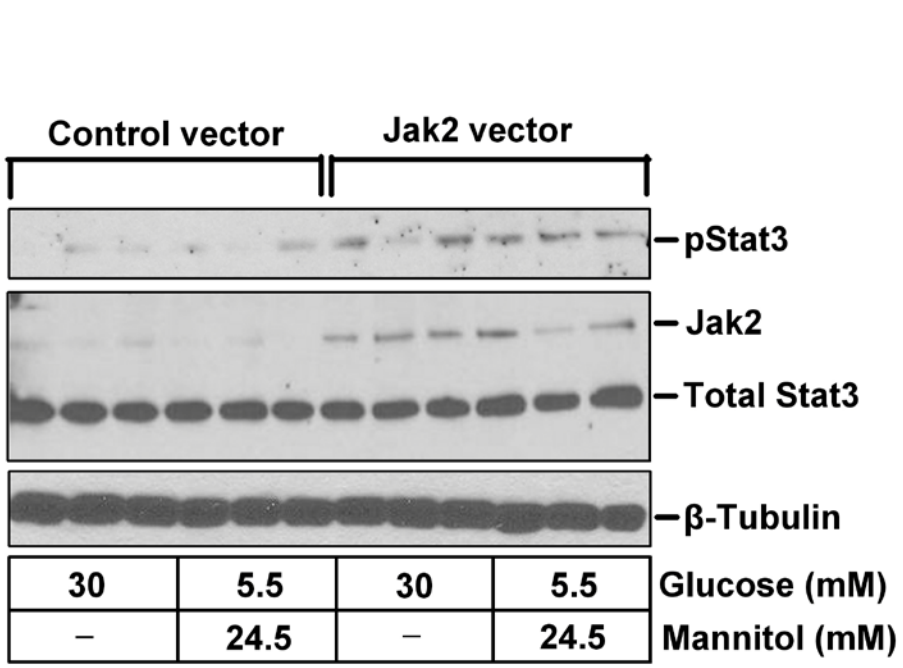
Berthier et al., Diabetes. 2009;58:469

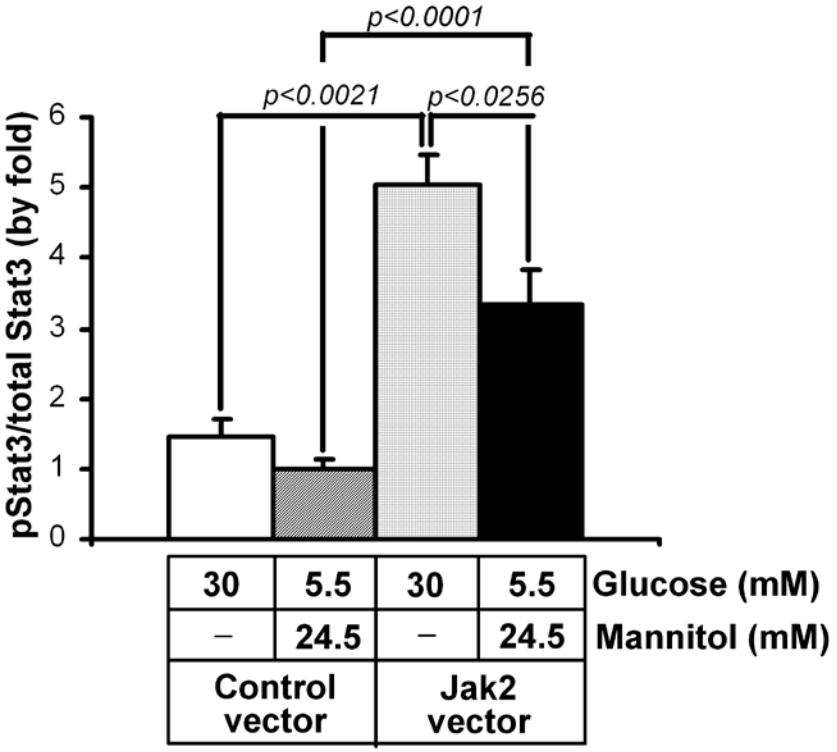
Jak2 in Human Progressive DN and non-diabetic Kidney Diseases



Berthier et al., Diabetes. 2009;58:469

Increased Stat3 phosphorylation with Jak2 overexpression





Berthier et al., Diabetes. 2009;58:469

JAK/STAT Pathways in Human Diabetic Nephropathy

Invited Review

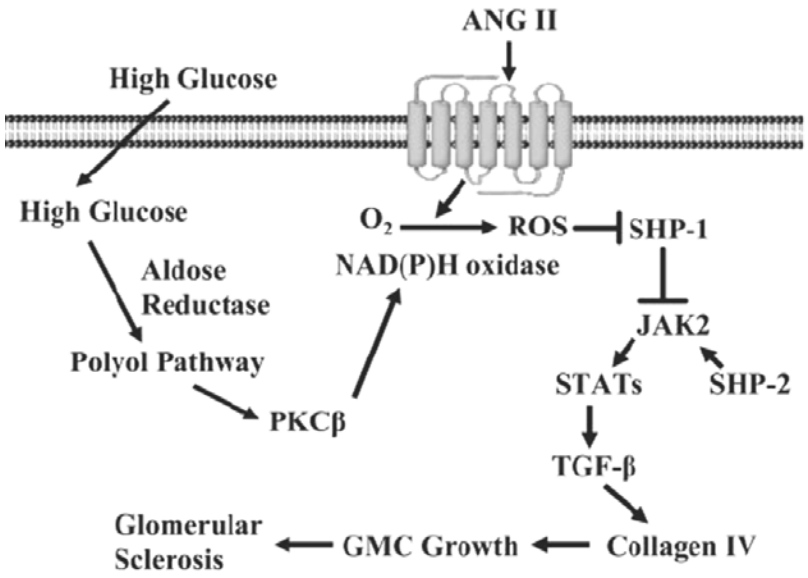
Am J Physiol Renal Physiol 290: F762–F768, 2006;
doi:10.1152/ajprenal.00181.2005.

Role of the JAK/STAT signaling pathway in diabetic nephropathy

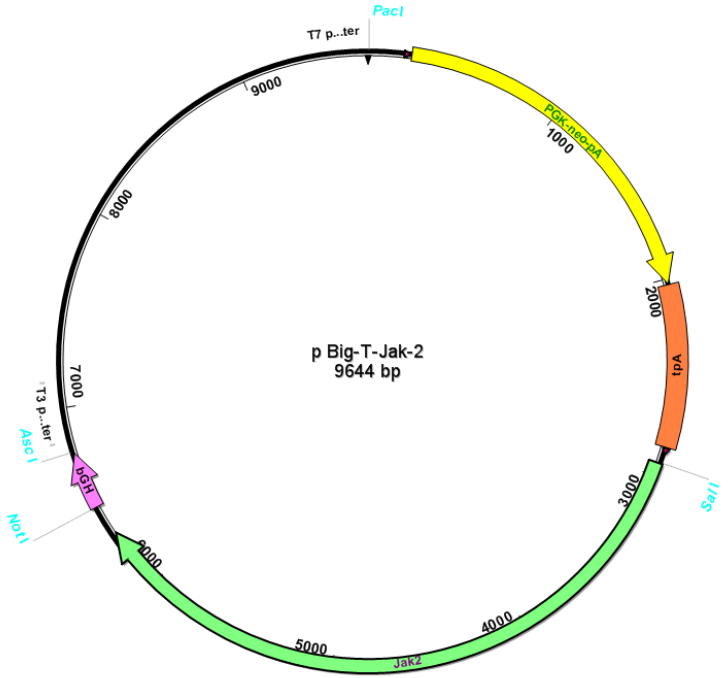
Mario B. Marrero,[1] Amy K. Banes-Berceli,[1] David M. Stern,[2] and Douglas C. Eaton[3]

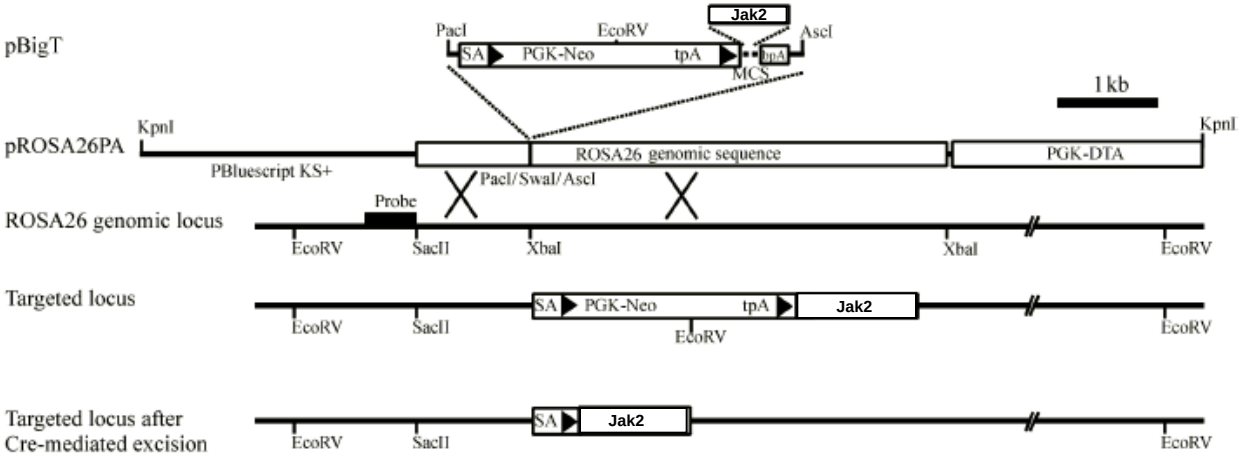
[1]*Vascular Biology Center and* [2]*Department of Medicine, Medical College of Georgia, Augusta; and* [3]*Center for Cell and Molecular Signaling and Department of Physiology, Emory University School of Medicine, Atlanta, Georgia*

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.

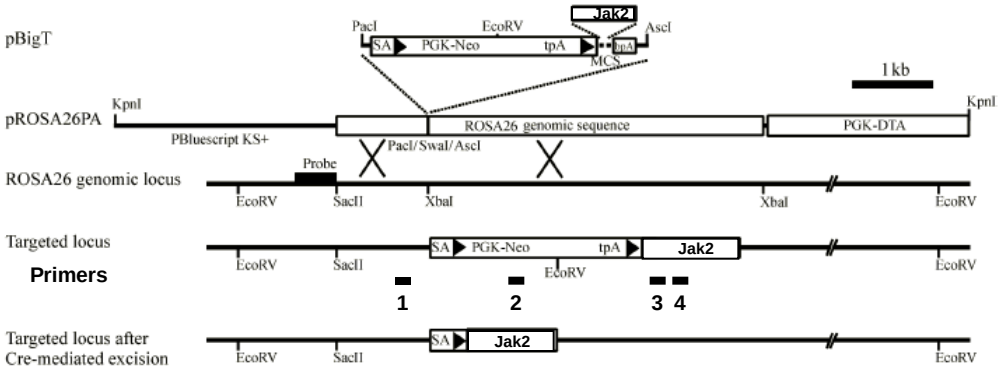


ROSA26/JAK2 Knockin 129S6/SvEvTac Mice

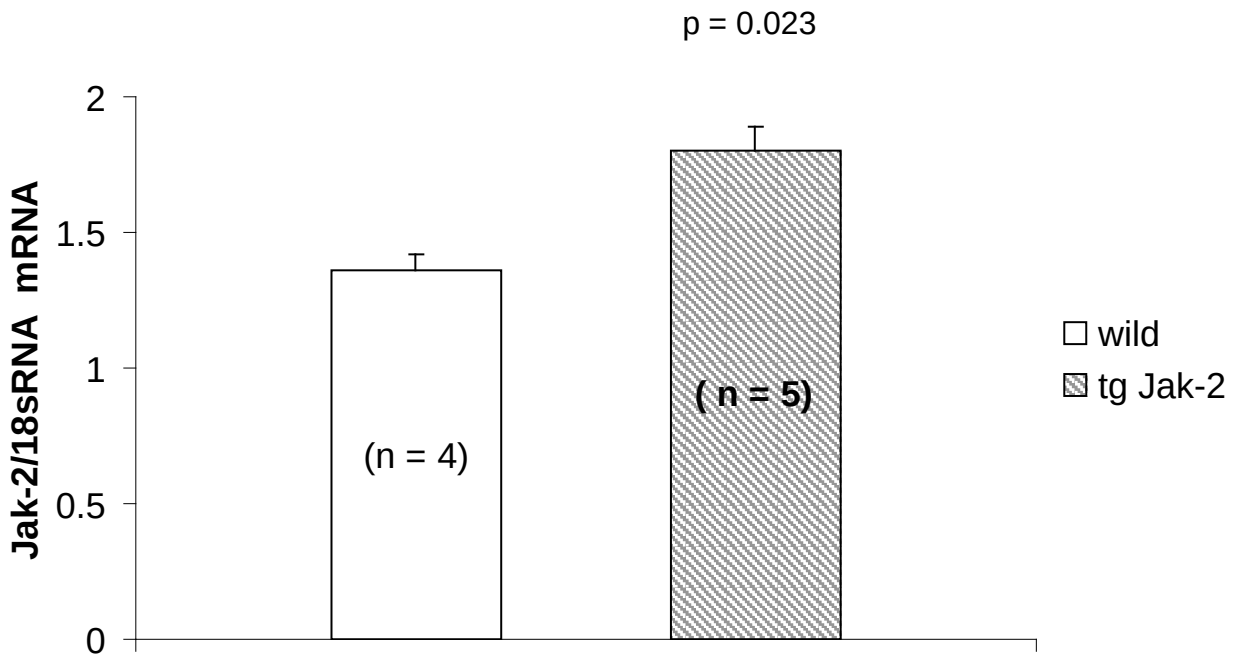




Cre recombinase activity on kidney cortex genomic DNA (females)

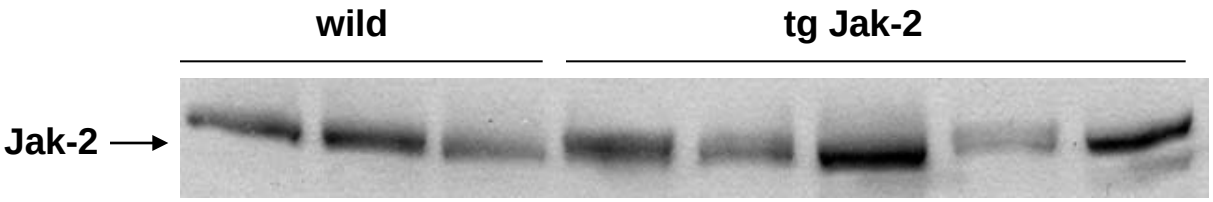


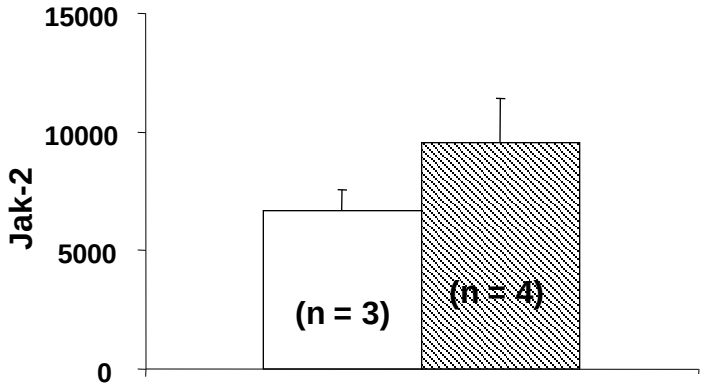
Jak-2 mRNA level (RT-PCR) in kidney cortex male PEPCK Cre[1]/Jak2 mice (4 weeks)



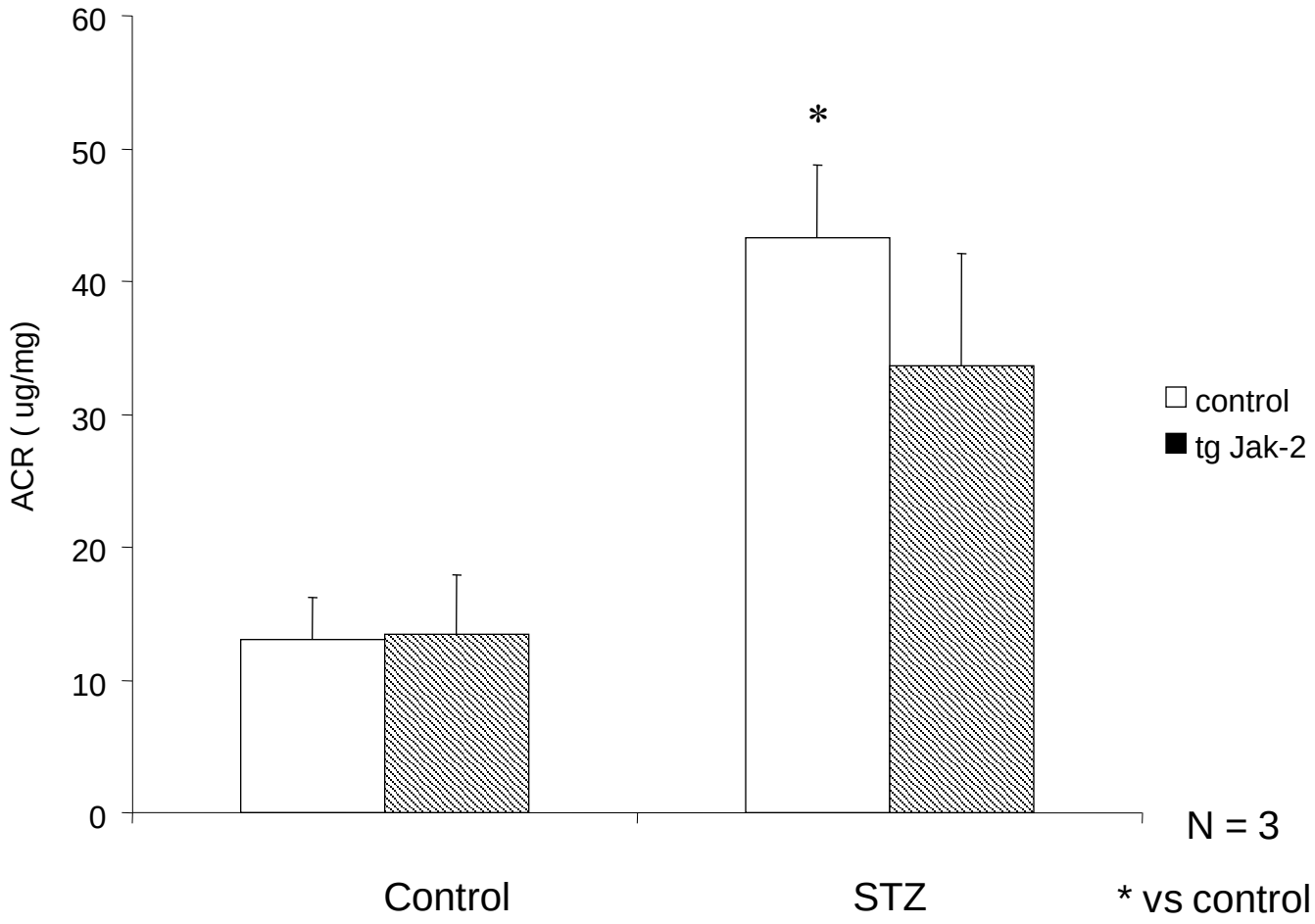
[1]Thanks to Susan Gurley and Tom Coffman

Jak-2 protein level in kidney cortex male PEPCK Cre/Jak2 mice (4 weeks)





ACR male PEPCK Cre/Jak2 mice (12 weeks)



From MGHC

- 129S6-stop-flox Jak2 stock
- 129S6-Podocin-cre stock

UM Nephropathy

- JAK2 and other JAK/STAT members are expressed at elevated levels in human diabetic nephropathy.
- A 129S6/SvEvTac stop/flox JAK2 mouse stock is now available from MGHC as is a 129S6/SvEvTac Podocin Cre mouse.
- PEPCK Cre/JAK2 mice show variable overexpression of JAK2 in kidney cortex at 4 wks of age.
- A trial of STZ diabetes is ongoing in PEPCK Cre/JAK2 mice and controls.
- A trail of STZ diabetes in Podocin Cre/JAK2 mice will be initiated shortly.

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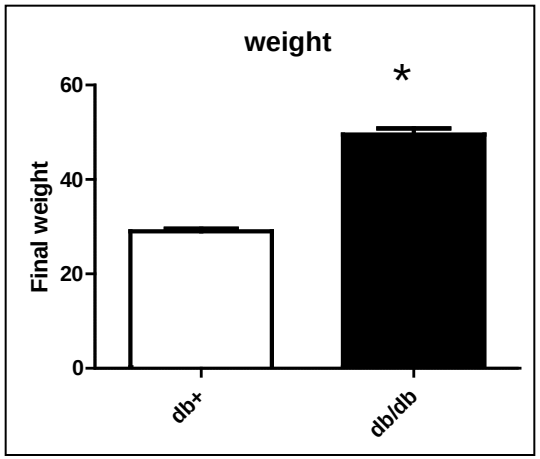
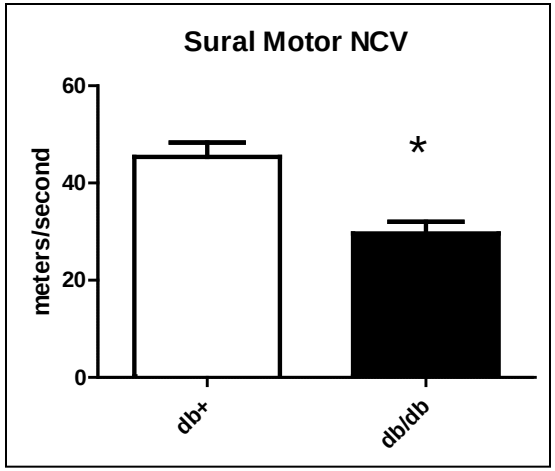
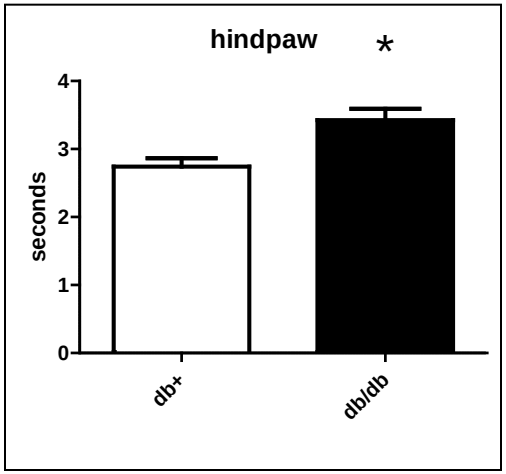
EVA FELDMAN: NEUROPATHY
Animal Models:
Completed Neuropathy Phenotyping

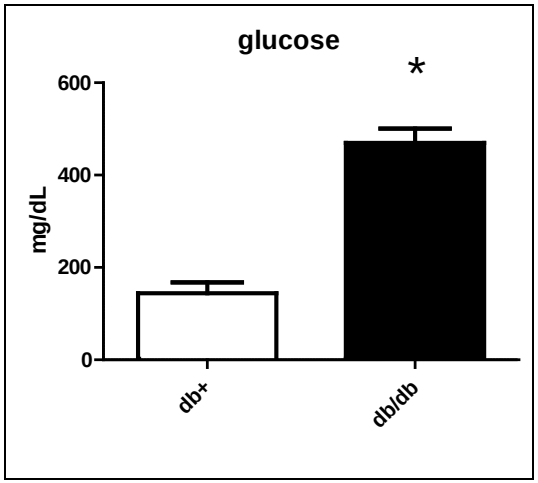
	Tail Flick Hind P aw every 8-12 wks	**NCV Measure d 12 or 16 and /or 24 wks post-diabetes**	**IEFD Measured at tissue harvest**	**Biochemistry Measured at tissue harvest**	**Advanced Neuropathy Phenotyping**
C57BLKS db/db	X	X	X	X	X
C56Bl6 +/- high fat diet	X	X	x	X	x
ApoE +/- db/db	X	Ongoing	Ongoing	Ongoing	NA
C56Bl6 SOD1 +/-	X	X	Ongoing	Ongoing	Ongoing
Denver Mice	NA	X	X	X	X
Akita STZ	X	X	X	X	X
Pdx +/+ and +/-	NA	X	X	X	X
KO Tg STZ	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing
DbA STZ ± feno fibrate	X	X	X	X	X

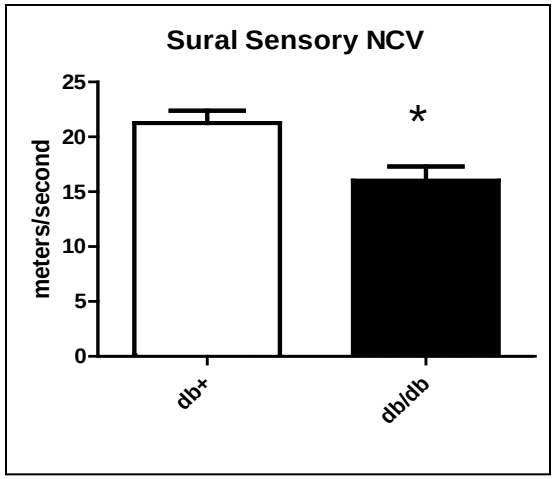
X = completed NA = not applicable

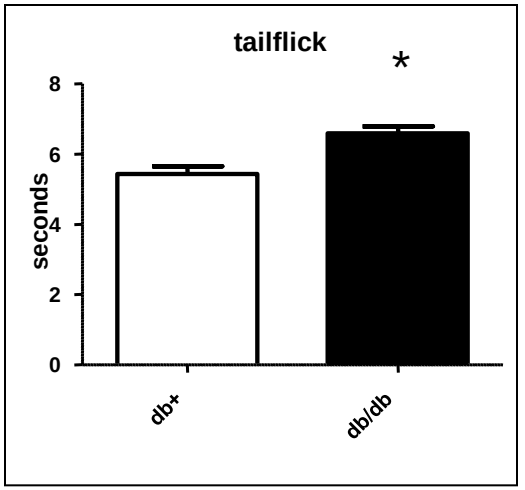












*: p-value < 0.001

- Blood glucose levels and weights were significantly higher in db/db mice.
- Sural nerve conduction velocities were significantly decreased in db/db mice.
- Hindpaw and tailflick were significantly increased in db/db mice.