TUBULAR OVEREXPRESSION OF GREMLIN IN TRANSGENIC MICE AGGRAVATES RENAL DAMAGE IN DIABETIC NEPHROPATHY

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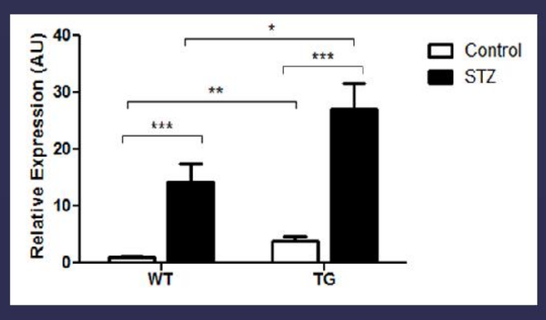
ABSTRACT

Diabetic nephropathy (DN) is currently a leading cause of end stage renal failure worldwide. Gremlin is a glycoprotein that acts as a mediator of TGF-β and Smad signaling pathway activation and was identified as a gene differentially expressed in mesangial cells exposed to high glucose and it has been shown that allelic Gremlin depletion attenuates experimental diabetic kidney disease. We have described that Gremlin is highly expressed in biopsies from patients with diabetic nephropathy, predominantly in areas of tubulointerstitial fibrosis and co-localized with TGF-β suggesting a role for Gremlin in this nephropathy. To study the *in vivo* role of Gremlin in DN, we developed a streptozotocin (STZ) diabetic model in transgenic mice expressing human Gremlin in proximal tubular epithelial cells.
In this experimental model, mice developed blood glucose between 300 and 500 mg/dl. The albuminuria /creatinuria rate, determined at week 20 was significantly increased in the diabetic animals, but no significant differences between transgenic (TG/STZ) and wild type (WT/STZ). To assess the level of kidney damage, renal tissue was analyzed by light microscopy (PAS and Masson staining,), electron microscopy, IHC and qPCR. At glomerular level, TG/STZ mice had significant thickening of the basement membrane, increased mesangial matrix and podocytopenia versus WT/STZ mice. At tubulointerstitial level, TG/STZ animals showed increased cell infiltration and mild interstitial fibrosis. In addition, we observed a decrease expression of Podocin and increased expression of inflammatory and fibrosis markers. Together, these results show that transgenic mice overexpressing Gremlin in renal tubules develop greater glomerular and tubulointerstitial injury in diabetic nephropathy. FONDECYT 1120480

METHODS and RESULTS

1.- Animals: We used transgenic (TG) mice expressing GREM1 in the renal proximal tubular cells under the control of a specific kidney androgen-regulated promoter (KAP) as we recently report (PLoS One. 2014 Jul 18;9(7):e101879).



2.- Induction of Experimental Diabetes in mice: Diabetes was induced in 15 wild type (WT) and 36 TG mice with STZ (50 mg/kg) on five consecutive days. Blood glucose levels were checked once a week. Diabetic animals were treated three times weekly with 0.4 U insuline from week 16 until the week 25.

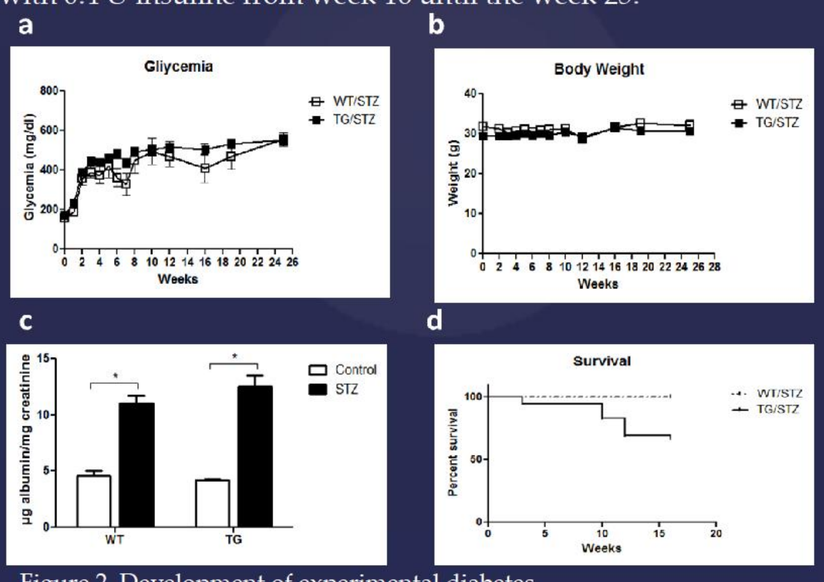
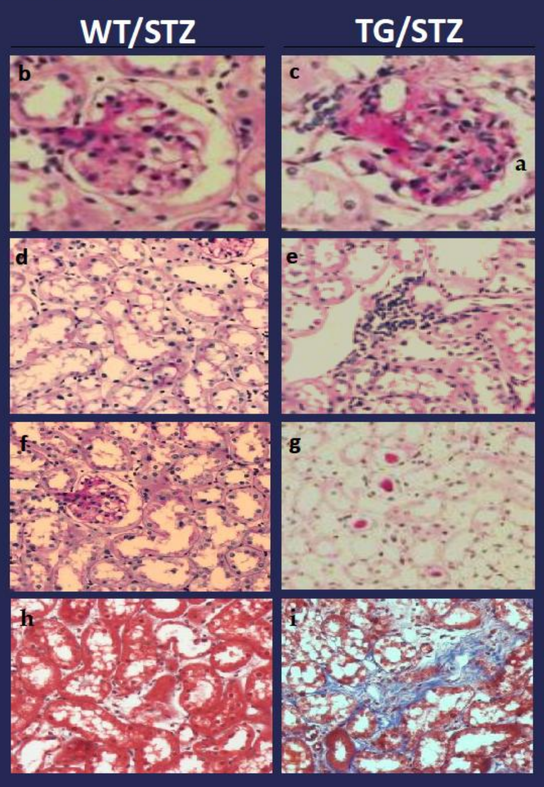


Figure 2. Development of experimental diabetes.

3. Histochemical analysis of renal tissue of diabetic animals: Tubular and interstitial lesions were graded from 0 to 4 and analyzed as a histopathological score with PAS and Masson Trichrome staining) (Zoja et al. JAmSocNephrol 2002).



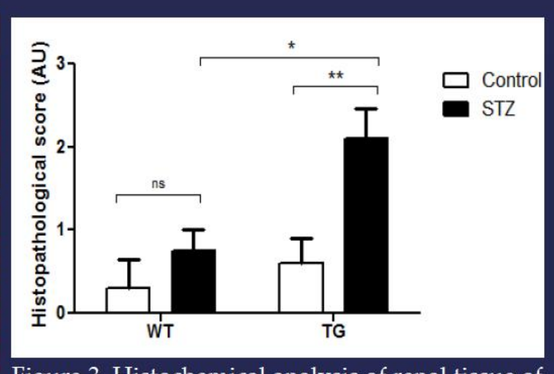


Figure 3. Histochemical analysis of renal tissue of diabetic animals. a) Histopathological score at week 25. Representative images of kidney sections of WT/STZ and TG/STZ mice, stained with PAS (b-g) and Masson (h-i). PAS staining showed glomerular more mesangial increase and hyalinosis (3c), cellular infiltration (3e) and presence of hyaline casts in the renal tubules of animals TG/STZ (3f). Increased interstitial fibrosis was demonstrated in TG/STZ using Masson (3h-i).

4.- Ultraestructural analysis of renal tissue of diabetic mice

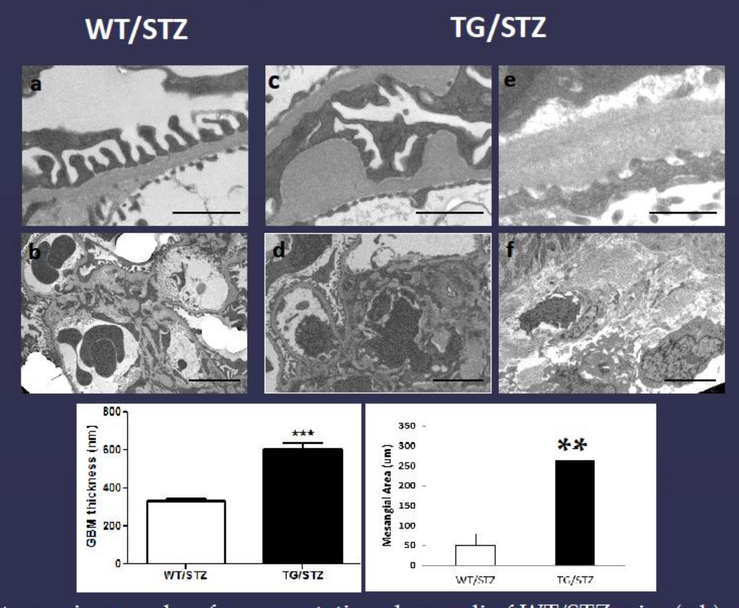


Figure 4. Electron micrographs of representative glomeruli of WT/STZ mice (a,b) and TG/STZ (c-f) showing GBM thickening (a, c, e); mesangium (b,d); and infiltrating inflammatory cells in the interstitium (f). Bars 1 um; 5 um. Quantitative measure of GMB thickness (g) and mesangial area (h). The results are expressed as the mean ± SE. **p<0.01, ***p<0.001,

5. Podocyte density analysis: Sections were incubated with Monoclonal Mouse Anti-Human Wilm's Tumor 1 (WT1) followed by incubation with the M.O.M. Immunodetection kit and DAB Peroxidase Substrate

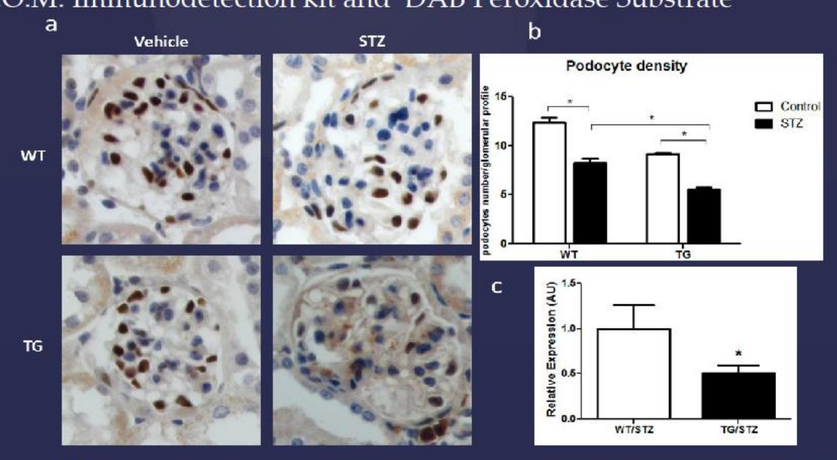


Figure 5. Podocyte density and podocin gene expression in kidneys of diabetic and control animals a) Representative images of podocyte marker WT-1. b) Graph of average number of podocytes per glomerular profile observed of a total of 25 glomeruli per animal c) Podocin. gene expression

6. Podocyte Gremlin Expression in kidneys of transgenic diabetic mice

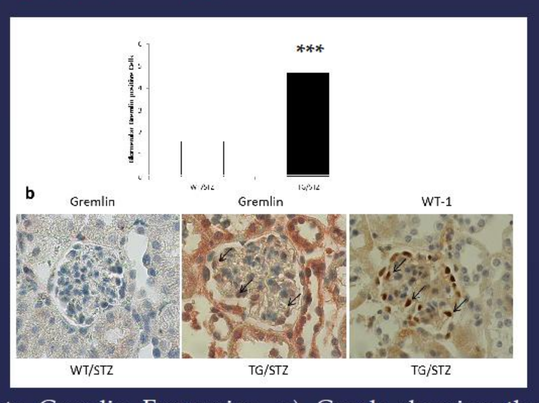


Figure 6 Podocyte Gremlin Expression. a) Graph showing the average number of glomerular Gremlin positive cell observed of a total of 25 glomeruli per animal b) Representative images of IHC against podocyte marker WT-1 (brown stained nuclei) and against Gremlin (red stained) in serial cuts of renal tissue of TG/STZ mice.

7. Gene expression of fibrotic and inflammatory markers in renal tissue of diabetic mice

Figure 6. Expression analysis by qPCR. Analysis of a) Tgf-β, b) col1a1, c) Fn1, d) αSma, e) Mcp1 and e) Opn were performed by qPCR from total renal RNA samples from WT/STZ=13 and TG/ STZ=22. mice.

CONCLUSION

These results show that transgenic mice overexpressing Gremlin develop greater diabetic renal damage and suggests that Gremlin plays a role in the development of glomerular and tubulointerstitial injury. Fondecyt 1120480 Chile

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