Tubular overexpression of Gremlin in transgenic mice aggravates renal damage in diabetic nephropathy

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DIABETIC NEPHROPATHY (DN) is currently the leading cause of end-stage renal failure worldwide. Gremlin was identified as one of the developmental genes induced in cultured human mesangial cells exposed to high glucose (24), and it is differentially expressed in kidneys of diabetic rodents (47) and in patients with diabetic nephropathy (10, 23). Gremlin is a secreted member of the cysteine knot superfamily, belongs to a family of bone morphogenetic proteins (BMPs) antagonists, and is highly conserved during evolution (25, 45). Several studies have demonstrated that Gremlin is a BMP antagonist that interacts with these proteins, in particular with BMPs -2, -4, and -7, blocking their bindings to their specific receptors, and regulates downstream related processes, including nephrogenesis, fibrosis, and cancer (3, 7, 16, 17, 27). However, many data show that Gremlin can exert BMP-independent responses, including the regulation of cell migration and angiogenesis in endothelial cells, and the activation of Smad signaling and related profibrotic effects in tubular epithelial cells (37, 42, 46). In this way, in endothelial cells Gremlin binds to vascular endothelial growth factor receptor-2 (VEGFR2), the main transducer of VEGF-mediated angiogenic signals, in a BMP-independent manner (26). In tubular epithelial cells, Gremlin also binds to VEGFR2 independently of BMPs antagonism (20). Moreover, in the murine kidney, Gremlin via the VEGFR2 signaling pathway induces an inflammatory response (20).

We and other authors have suggested that Gremlin could be considered a mediator of renal injury in diabetic nephropathy (10, 18, 48). In experimental mice models of streptozotocin (STZ)-induced diabetes, Gremlin deletion, using heterozygous mice for the Grem1 gene or by a small interfering (si) RNA gene silencing-based approach, ameliorated renal damage (39, 50). Many studies suggest that podocyte damage by a high-glucose environment is the main cause of diabetic-induced renal injury, being that podocyte loss by apoptosis is an important event in the onset of proteinuria (1, 32, 40, 41). An in vitro study has shown that Gremlin gene silencing can prevent high glucose-induced podocyte apoptosis (22), explaining, at least in part, the beneficial effects of Gremlin blockade found in experimental diabetic models. In human biopsies of diabetic nephropathy, we have noted that Gremlin is highly expressed, predominantly in areas of tubulointerstitial fibrosis and colocalized with transforming growth factor-β (TGF-β) (10). In addition, Gremlin mRNA levels directly correlate with elevated serum creatinine levels and tubulointerstitial damage (6). Moreover, we have recently shown that
recombinant Gremlin directly activates cultured renal fibroblasts and tubular epithelial cells, inducing profibrotic-related events and acts as a downstream profibrotic mediator of TGF-β-actions (36). Therefore, all of these data suggest that Gremlin could contribute to tubulointerstitial fibrosis by direct activation of tubulointerstitial cells. Recently, our group generated a transgenic (TG) mouse expressing human Gremlin specifically in renal proximal tubular cells under the control of an androgen-regulated promoter (11). This tubular Gremlin overexpression generates no functional abnormalities or renal damage under physiological conditions, but exerts increased susceptibility to folic acid-induced acute renal injury (11). In diabetic nephropathy, besides podocyte damage, the progression to end-stage renal failure is related to tubulointerstitial fibrosis. Our aim was to investigate whether specific Gremlin overexpression in tubular cells could contribute to the onset and progression of diabetic-induced renal damage.

MATERIALS AND METHODS

Animals. A TG mouse line expressing the human Gremlin-1 gene (GREM1) was generated at the mouse facility of the Centro de Estudios Científicos-CECs, Chile, and previously characterized (11). We used line A TG mice expressing the human Gremlin gene in renal proximal tubular cells under the control of a specific kidney androgen-regulated promoter (KAP), screened using PCR with the following primers: GREM1 intron 1F (5'-GCCGATAGGAAATTCTCTAATAGG-3'), KAP promoter F (5'-ATGAGCACTTAA TGGCTAGT-3'), and GREM1 exon 2R (5'-TCCAAATCGATGGA TGCAAC-3') as we reported recently (11).

TG and C57Bl6/J wild-type (WT) mice, the latter used as a control, were subjected to a 12:12-h light-dark cycle with access to food and water ad libitum. Principles of laboratory animal care were followed, and euthanasia of the mice was done with administration of anesthesia, following the protocols approved by the Committee on the Ethics of Animal Experiments of Universidad Austral de Chile (Permit No. 20.2011), the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) Ethics Committee, and according to National Institutes of Health guidelines.

Model of experimental diabetic nephropathy in mice. The model of diabetic nephropathy was induced by STZ administration as previously described (2, 5, 34). Diabetes was induced in 15 WT and 36 TG mice by 30 mU/kg STZ (Sigma, St. Louis, MO) dissolved in vehicle (0.05 M citrate buffer, pH 4.5) within 10 min of preparation by 5 consecutive days (28). Nondiabetic TG and WT control animals were injected with vehicle only. Blood glucose levels were checked before and during administration of STZ and once a week with Accu-chek Performa (Roche, Mannheim, Germany). To keep body weight and prevent ketoacidosis and mortality, the diabetic animals were treated three times per week since week 16 after treatment with 0.4 U insulin (Humulin, Eli Lilly, Indianapolis, IN). To reduce the potential nephrotoxicity caused by STZ and maintain blood glucose levels in a controllable range according to suggestions by Tesh and Allen (44), an attenuated dose of STZ was administered to TG and WT mice.

Spot urine samples were collected once a week from all mice and analyzed for albumin by ELISA (ALPCO Immunoassays, Salem, NH) and creatinine with a Creatinina Wiener Lab Kit (Wiener Lab, Rosario, Argentina).

At 25 wk after the STZ administration, mice were anesthetized with 2% 2,2,2-trichloroethanol (Sigma) dissolved in 2-methyl-butanol (Sigma). The kidneys were removed, decapsulated, and cut along the sagittal plane. A portion of the left kidney was fixed in 4% formaldehyde or 2% glutaraldehyde, while the right kidney was immediately frozen in liquid nitrogen and processed for RNA and protein extraction. The specimens were embedded in paraffin and cut into 4-μm tissue sections for further histological [periodic acid-Schiff (PAS)/Masson] and immunohistochemical (IHC) studies.

The tissue fixed in 2% glutaraldehyde (Merck, Darmstadt, Germany) was postfixed with 1% osmium tetroxide (Ted Pella, Redding, CA), embedded in resinst embedded in resin EMBed-812 (EMS, Hatfield, PA), cut, stained, and observed under an electron microscope (Philips Tecnai12 BioTWIN, Philips, Eindhoven, The Netherlands) at 80 kV.

Histological analysis and IHC. Glomerular and tubulointerstitial lesions were graded as a histopathological score from 0 to 4 as previously described by Zoya (51). In brief, glomerular (sclerosis, increased mesangial matrix, and hyalinosis), tubular (atrophy, casts, and dilatation), and interstitial changes (fibrosis and inflammation) were graded from 0 to 4+ (0, no changes; 1+, changes affecting <25% of the sample; 2+, changes affecting 25–50% of the sample; 3+, changes affecting 50–75% of the sample; and 4+, changes affecting 75–100% of the sample). IHC for detection of Wilms tumor (WT-1) protein as a podocyte marker was performed following heat-induced epitope retrieval (10 mM Tris-base, 1 mM EDTA, 0.05% Tween 20, pH 9.0) for 10 min in a pressure cooker (Oster). Sections were incubated overnight with Monoclonal Mouse Anti-WT-1 Protein Clone 6F-H2 (dilution: 1:100, Dako, Carpinteria, CA) followed by incubation with M.O.M. Immunodetection kit PK 2200 (Vector, Burlingame, CA) and ImmPACT DAB Peroxidase Substrate (Vector).

IHC for detection of Gremlin and TGF-β was performed following heat-induced epitope retrieval (microwaving for 10 min in citrate buffer). Murine Gremlin was detected using goat anti-murine Gremlin antibody (dilution 1:20, AF956, R&D Systems, Minneapolis, MN) overnight at 4°C with Immpress Reagent MP7405 (Vector), and the reaction was developed with ImmPACT Alcian Blue 280 (Vector). Murine TGF-β was detected with anti-murine TGF-β (Fitzgerald, Acton, MA) and Immpress Reagent Kit MP7401 (Vector), revealed with DAB SK4105 (Vector), and counterstained with hematoxylin.

Image analysis and quantification of the IHC signals were performed using the KS300 imaging system, version 3.0 (Zeiss). For each sample, the mean staining area was obtained by analysis of 20 fields (×20). The staining score is expressed as square millimeters per density. Podocyte density was calculated by enumerating podocyte nuclei stained for WT-1 and Gremlin-positive glomerular cells by counting stained cells by IHC in 25 glomeruli of 4 mice in each group.

Quantitative PCR gene studies. For mRNA isolation, frozen kidney pieces from WT control (n = 10), WT/STZ (n = 13), TG control (n = 9), and TG/STZ (n = 22) mice were pulverized in a metallic chamber and dissolved in 1 ml of TRIZol (Invitrogen, San Diego, CA). Total RNA was extracted with TRIZol according to the method provided by the manufacturer, treated with DNase I to remove potential contamination with genomic DNA, and reverse transcribed by using random primers and the Improm-II Reverse Transcription System (Promega, Madison, WI) to synthesize double-stranded cDNA. Quantitative (q) PCR was performed with the commercial reagent Kapa SYBR FAST Universal 2× qPCR Master Mix (Kapa Biosystems, Wilmington, MA) to determine the expression of genes of interest. Primers used for qPCR are shown in Table 1.

PCR product specificity was verified by melting curve analysis, and all of the real-time PCR reactions were performed in triplicate. The 2^-ΔΔCt method was used to analyze the relative changes in gene expression levels (21).

Statistical analysis. The results are expressed as means ± SE. A Mann-Whitney U-test was performed to compare all the tubular/interstitial lesions, IHC signals, and qPCR results in the TG and WT mice injected with STZ. Values of P < 0.05 were considered significant.
RESULTS

Increased renal damage by STZ-induced experimental diabetes in Gremlin TG mice. To determine the effect of tubular-specific Gremlin overexpression on the development of diabetes in vivo, a model of diabetes-induced renal damage by STZ administration was done in TG mice expressing human Gremlin in renal proximal tubular cells (11) compared with WT mice. After 2 wk of STZ administration, TG/STZ and WT/STZ were diabetic, maintaining serum glucose levels between 300 and 600 mg/dl for 25 wk after being treated (Fig. 1A). In addition, body weight was similar between TG/STZ and WT/STZ (Fig. 1B). At week 20, the urine albumin/creatinine ratio (ACR) was increased in both diabetic groups compared with healthy controls (P < 0.05), but with no significant differences between them (Fig. 1C). Control nondiabetic WT and TG mice did not show significant differences in glycemia (WT: 151.4 ± 9.99 vs. TG: 148.1 ± 4.31 mg/dl) and body weight (WT: 31.80 ± 1.03 vs. TG: 29.48 ± 0.92 g).

Interestingly, TG/STZ mice had higher mortality rates than WT/STZ (38.9% TG/STZ, 19.9% WT/STZ) (P < 0.05) as shown in a Kaplan-Meier analysis (Fig. 1D). It is worth noting, however, that TG control mice had similar survival rates to WT control mice until 24 mo.

Noteworthy, the histopathological analysis of diabetic mice showed a significantly higher tissue damage score in TG/STZ vs. WT/STZ (P < 0.05) (Fig. 2A), which was calculated by assessing the degree of glomerular sclerosis, increased mesangial matrix, hyalinosis, tubular casts, acute tubular damage, tubular atrophy, the presence of interstitial inflammatory cells, and interstitial fibrosis. As illustrated in Fig. 2, PAS staining showed a significantly higher increase in mesangial matrix and hyalinosis (Fig. 2C), interstitial cellular infiltration (Fig. 2E), and the presence of hyaline casts (Fig. 2G) in the renal tubules of TG/STZ compared with WT/STZ mice (Fig. 2, B, D, and F). Moreover, Masson’s trichrome staining showed higher interstitial fibrosis in TG/STZ vs. WT/STZ mice (Fig. 2, H and I).

To evaluate further the glomerular damage, an electron microscopy analysis was done. Diabetic mice showed thickening of the glomerular basement membrane (GBM) that was significantly higher in TG/STZ than WT/STZ mice (Fig. 3G). Additionally, in TG/STZ mice the GBM presented irregular laminations and localized protrusions in some segments, along

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**Table 1. Primers used for qPCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>GREM1</td>
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<td>5'-GGGAGGAGAAGGATGTGGG-3'</td>
</tr>
<tr>
<td>Tgf-β1</td>
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<td>5'-CTGTCGACCTGCGGTTGCG-3'</td>
</tr>
<tr>
<td>αSma</td>
<td>5'-TCCTGACATGACCTGACAG-3'</td>
<td>5'-AGAGATCGATGATCGGTGAC-3'</td>
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<tr>
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<td>5'-CGATCCAGTCGGCTGGTTCC-3'</td>
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<tr>
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<td>5'-ACCCACGACCCACTCCTTT-3'</td>
<td>5'-GCTTCCCTGCTGCTCTCTCC-3'</td>
</tr>
<tr>
<td>Mcp1</td>
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<td>5'-GGGTTAACTGCTCTGCTC-3'</td>
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<tr>
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<td>Cyc</td>
<td>5'-GGCAATGCTGGCACAACAA-3'</td>
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See the text for definitions.

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**Fig. 1. Development of experimental diabetes.** Evolution of glycemia (A), body weight (B), albumin/creatinine ratio (ACR; C), and survival (D) of wild-type (WT) and transgenic (TG) mice treated with streptozotocin (STZ) followed for 25 wk. At week 0, WT/STZ (n = 15) and TG/STZ (n = 36) are shown. Glycemia, ACR, and weight are expressed as means ± SE. The survival results are presented as percentage of cumulative survival and were estimated and analyzed by the Kaplan-Meier method. The P value was calculated according to a log-rank test.

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with greater foot process effacement (Fig. 3, C and E). Furthermore, a significant increase in mesangial matrix (Fig. 3, D and H) and inflammatory cells in the interstitium was found (Fig. 3F). GBM and mesangial area of control WT mice are shown in Fig. 3, A and B, respectively. The morphometric ultrastructural scores of GBM thickening and increased mesangial matrix of four animals in each group are shown in Fig. 3, G (P < 0.001) and H (P < 0.01).

All these data clearly demonstrate that STZ-mediated diabetic nephropathy caused greater tissue damage in the tubular-specific GREM1 TG mice than in WT mice.

Podocyte density analysis. To assess the number of podocytes in the glomeruli of diabetic mice, IHC against WT-1, used as a podocyte marker protein, was performed. It was observed that TG mice exhibit a mild significant decrease in the number of podocytes in relation to WT mice (Fig. 4, A and B). However, in diabetic mice, podocyte number was strongly decreased, mainly in the TG/STZ group vs. WT/STZ (P < 0.05). Additionally, the expression of podocin was significantly decreased in diabetic TG mice (P < 0.05) compared with WT (Fig. 4C). Therefore, these results suggest that this podocyte loss was both genotype and diabetes dependent.

To analyze whether these podocytes could express Gremlin, we performed specific IHC for each protein in serial sections of the same renal tissue (Fig. 5). TG/STZ mice had a significant increase in the number of positive Gremlin-expressing cells (P < 0.001) (Fig. 5A), and the majority of these cells corresponded to podocytes (Fig. 5B).

Gremlin gene expression analysis. To confirm the activity of the transgene, Gremlin expression was evaluated in TG and WT mice by qPCR. According to our previous results, we found Gremlin expression significantly increased in TG vs. WT mice (3.78 ± 0.85 vs. 1.00 ± 0.29, P < 0.01). Due to the high homology (98%) between the sequences of human Gremlin and endogenous murine Gremlin (Grem1), the primers used in the qPCR recognize both sequences, allowing evaluation of the expression of endogenous Gremlin in response to induced diabetes.

After STZ administration and once the mice were diabetic, both TG/STZ and WT/STZ groups presented a significant increase in renal Gremlin expression (P < 0.001); that finding was significantly higher in TG/STZ (P < 0.05) (Fig. 6).

Increased renal profibrotic and proinflammatory genes in STZ-induced experimental diabetes in Gremlin TG mice. To evaluate the contribution of specific Gremlin tubular overexpression in STZ-induced renal fibrosis, several profibrotic and matrix-related genes were evaluated by real-time PCR. Renal gene expression of Tgfb1 (P < 0.001) (Fig. 7), type I procollagen (Col1a1) (Fig. 7), fibronectin (Fn1) (Fig. 7), and α-smooth muscle actin (α-SMA; αSma) (Fig. 7) was significantly increased in TG/STZ vs. WT/STZ (P ≤ 0.05). Furthermore, we detected more intense tubular expression of Gremlin and TGF-β protein in TG/STZ vs. WT/STZ mice (Fig. 8).

Additionally, we analyzed the gene expression of two proinflammatory molecules, MCP (Mcp1) and osteopontin (Opn), involved in early activation of lymphocyte T. Mcp1 was
significantly increased in TG/STZ compared with WT/STZ (Fig. 7); however, no significant changes were observed in Opn gene expression (Fig. 7).

DISCUSSION

Despite the intensive research done in the field of diabetic nephropathy, our current armamentarium for the treatment of this disease is still inadequate (14). Emerging evidence suggests that Gremlin could be an important new therapeutic target for diabetic nephropathy. Earlier data showed that Gremlin is highly expressed in a high-glucose environment in vitro (24) and in biopsies from patients with diabetic nephropathy (10). Experimental data have demonstrated beneficial effects of Gremlin inhibition both in vitro (33, 37) and in vivo (39, 50) in diabetic kidney disease. In this study, we found that specific Gremlin overexpression in tubular cells increased tubulointerstitial damage and fibrosis in response to STZ-induced diabetic renal injury, supporting the hypothesis that Gremlin is a deleterious factor contributing to the progression of renal damage in diabetic nephropathy.

We observed that the onset of experimental diabetes was similar between Gremlin TG and WT. Both groups developed hyperglycemia within the first 2 wk. Despite high levels of glycemia, no significant changes in body weight or ACR between TG/STZ and WT/STZ mice were observed, with basal values of ACR similar to those described previously (11). ACR values in diabetic mice were lower than those described by other authors in models of STZ (ACR 30–70 μg/mg) (34), possibly reflecting a reduction in direct renal toxicity by using an attenuated dose of STZ. Furthermore, it is known that the mouse strain C57BL6/J, although having a high susceptibility to developing diabetes (8), is characterized by being relatively resistant to developing albuminuria and kidney damage than other strains such as 129Sv and DBA/3J, among others (34).
An important finding was that, despite insulin treatment, there was a marked increase in mortality in Gremlin TG mice, suggesting a deleterious role of Gremlin in diabetic mice.

Previous studies have characterized the histological features of diabetic nephropathy in C57BL/6J mice. Between 15 and 30 wk of hyperglycemia, mesangial matrix expansion has been found. However, other characteristics of diabetic nephropathy, such as glomerulosclerosis, arteriolar hyalnosis, mesangiopathy, and podocytopenia, are rare in this strain (4). In agreement, histological analysis of WT/STZ mice showed mild or no damage at all, as observed in control nondiabetic animals. On the other hand, TG/STZ mice presented significant mesangial matrix increase, arteriolar hyalnosis, tubular hyaline casts, and incipient interstitial fibrosis, although no glomerulosclerosis or acute tubular damage was observed. At the ultrastructural level, both WT and TG diabetic mice showed GBM thickening, foot process effacement, increase in mesangial matrix, and the presence of infiltrating cells in the interstitium. These features were more severe in TG/STZ mice; additionally, we observed an irregular thickening, localized protrusions, and laminations in the GBM of TG/STZ mice. These results suggest that overexpression of Gremlin in a hyperglycemic environment effectively influences the development of renal damage in vivo and accelerates diabetic nephropathy.

Podocytes are key cells in the pathogenesis of diabetic nephropathy (1, 32, 40, 41, 49) and chronic kidney diseases (41). Urinary excretion of podocytes was found in patients with diabetic nephropathy (29), and podocytopenia has been associated with progressive glomerular damage (31). Among other mechanisms, reactive oxygen species production by high glucose concentrations could mediate the loss of podocytes by apoptosis (12, 43). Podocyte injury or loss will lead to proteinuria, and it is associated with glomerular sclerosis (32). In TG and WT diabetic mice, a significant decrease in podocytes was observed, mainly in TG mice. Similarly, downregulation of podocin gene expression in TG/STZ mice was consistent with the podocytopenia observed. Noteworthy, we have observed Gremlin induction in advanced human diabetic nephropathy (10), and podocytic Gremlin expression could induce apoptosis (21). Moreover, in vitro studies in murine podocytes have shown attenuation of hyperglycemia-induced podocyte apoptosis by Gremlin gene silencing (22). In these TG/STZ mice, we demonstrate a significant increase in Gremlin expression also in podocytes, which could explain the stronger podocytopenia observed in these animals, not explained only for hyperglycemia as observed in the WT/STZ mice. Since a direct effect of human Gremlin on apoptosis in mouse podocytes has not been demonstrated, this could be a limitation of our study, although it is well known that human and murine Gremlin mRNA and protein exhibit high homology (89 and 98%, respectively).

During diabetic damage to the kidney, there is an upregulation of profibrotic factors that could contribute to renal fibrosis. In vitro studies have shown that TGF-β1, known as a major fibrogenic factor, regulates Gremlin expression (13, 24), and
Gremlin itself induces TGF-β1 production (22, 36). Therefore, in pathological conditions, both proteins could exert a bidirectional positive feedback, contributing to renal fibrosis. Accordingly, in TG/STZ mice TGF-β1 and Gremlin expression was significantly higher than in WT/STZ mice. Previous studies in cultured renal cells, including mesangial cells, podocytes, fibroblasts, and tubulointerstitial cells, have described that Gremlin regulates other profibrotic factors, including connective tissue growth factor (CTGF) (36, 38) and fibroblast growth factor (FGF) (19), increases the production of extracellular matrix proteins, and induces tubuloepithelial-to-mesenchymal transition (6, 36, 37). The analysis of specific fibrotic markers in Gremlin TG/STZ mice showed significant inductions of the extracellular matrix components (ECM) fibronectin and collagen type 1, as well as SMA, a marker of fibrotic phenotype and activated fibroblasts. This increase in ECM components forms the molecular basis for the presence of increased mesangial matrix, GBM thickening, and tubulointerstitial fibrosis, observed in TG diabetic mice. It is well known that GBM and the mesangial matrix are essentially constituted of collagen, proteoglycans, and glycoproteins such as fibronectin (30), and an increase in these components is directly related to their thickening. Our data support the idea that Gremlin could contribute to renal fibrosis by the upregulation of profibrotic factors and extracellular matrix components in the diabetic-damaged kidney. In addition, data presented here using a TG mouse strain

Fig. 5. Podocyte Gremlin expression in kidneys of TG diabetic mice at 25 wk. A: graph showing the average number of glomerular Gremlin-positive cells observed in a total of 25 glomeruli/animal; n = 4 for WT/STZ and n = 4 TG/STZ. B: representative images of immunohistochemistry against podocyte marker WT-1 (brown-stained nuclei) and against Gremlin (red stained) in serial sections of renal tissue of TG/STZ mice (arrows). Magnification ×630. Values are means ± SE. ***P < 0.001 by Mann-Whitney U-test.

Gremlin AGGRAVATES RENAL DAMAGE IN DIABETIC NEPHROPATHY

Fig. 6. Renal Gremlin expression in diabetic mice. Expression analysis was performed by quantitative PCR from total RNA samples from kidney of animals in each group. Control WT, n = 10; WT/STZ, n = 13; control TG, n = 9; and TG/STZ, n = 22. Values obtained were normalized by cyclophilin 1 expression levels (2^(-ΔΔct) method) and are presented as means ± SE. AU, arbitrary units. *P < 0.05, **P < 0.01, ***P < 0.001 by Mann-Whitney U-test.
that specifically overexpressed Gremlin in tubular cells supports the hypothesis by which tubular epithelial cell injury can result in renal fibrosis, including GBM thickening (15, 21), and therefore can play an important role in the development of chronic kidney disease.

However, numerous questions still remain unanswered. Although we may suggest that the increased glomerular Gremlin expression, mainly in podocytes of diabetic mice, could be a consequence of the hyperglycemic state, we cannot exclude that the overexpression of Gremlin in proximal tubules of TG mice might cause an upstream effect on glomerular cells.

Finally, in our study, molecular analysis indicated increased expression of the proinflammatory marker MCP-1, associated with the presence of inflammatory cell infiltration observed in TG/STZ mice. Furthermore, we have recently described that
Gremlin administration to mice induces the presence of inflammatory cells in the kidney and upregulates proinflammatory genes, including MCP-1 (20), extending previous in vitro studies in endothelial cells showing proinflammatory gene upregulation, closely related to its proangiogenic response (9).

In conclusion, the results obtained here provide evidence in vivo of the role of Gremlin in diabetic kidney disease by showing that overproduction of this protein confers increased susceptibility to developing renal damage and fibrosis in diabetic nephropathy in mice.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


