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Cellular Basis of Diabetic Nephropathy: V. Endoglin Expression Levels and Diabetic Nephropathy Risk in Patients with Type 1 Diabetes

Patricia Alvarez-Muñoz[1], **Michael Mauer, MD**[2,3], **Youngki Kim, MD**[3], **Stephen S. Rich, Ph.D.**[4], **Michael E. Miller, Ph.D.**[5], **Gregory B. Russell, M.S.**[5], **José M. Lopez-Novoa, PhD**[1], and **M. Luiza Caramori, MD, PhD**[2,*]

[1]Department of Physiology and Pharmacology, University of Salamanca, Spain

[2]Department of Medicine, University of Minnesota, Minnesota, USA

[3]Department of Pediatrics, University of Minnesota, Minnesota, USA

[4]Department of Public Health Sciences, University of Virginia, Virginia, USA

[5]Department of Biostatistical Sciences, Wake Forest University School of Medicine, North Carolina, USA

Abstract

Endoglin is an accessory receptor molecule that, in association with transforming growth factor β (TGF-β) family receptors types I and II, binds TGF-β1, TGF-β3, activin A, bone morphogenetic protein (BMP)-2 and BMP-7, regulating TGF-β dependent cellular responses. Relevant to diabetic nephropathy, endoglin, expressed in vascular endothelial and smooth muscle cells, fibroblasts, and mesangial cells, negatively regulates extracellular matrix (ECM). The aim of this study was to evaluate endoglin expression in cultured skin fibroblasts from patients with type 1 diabetes with and without diabetic nephropathy. Kidney and skin biopsies were performed in 125 type 1 diabetic patients. The 20 with the fastest rate of mesangial expansion (estimated by electron microscopy) and proteinuria ("fast-track") and the 20 with the slowest rate and normoalbuminuria ("slow-track"), along with 20 controls were studied. Endoglin mRNA expression was assessed by microarray and QRT-PCR and protein expression by Western blot. Age and sex distribution were similar among groups. Diabetes duration was similar (20±8 *vs.* 24±7 years), HbA_{1c} lower (8.4±1.2 *vs.* 9.4±1.5%), and glomerular filtration rate higher (115±13 *vs.* 72±20 ml/min/1.73m^2) in "slow-track" *vs.* "fast-track" patients. Microarray endoglin mRNA expression levels were higher in "slow-track" (1516.0±349.9) than "fast-track" patients (1211.0±274.9; p=0.008) or controls (1223.1±422.9; p=0.018). This was confirmed by QRT-PCR. Endoglin protein expression levels correlated with microarray (r=0.59; p=0.044) and QRT-PCR (r=0.61; p=0.034) endoglin mRNA expression. These studies are compatible with the hypothesis that "slow-track" type 1 diabetic patients, strongly protected from diabetic nephropathy, have distinct cellular behaviors that may be associated with reduced ECM production.

Keywords

diabetic nephropathy; type 1 diabetes; endoglin

Corresponding Author: M. Luiza Caramori, MD, PhD, 420 Delaware Street S.E., Mayo Mail Code 101, Minneapolis, MN 55455, Phone: (612) 624-5150, FAX: (612) 626-3133, caram001@umn.edu.

INTRODUCTION

TGF-β1 plays a pivotal role in the extracellular matrix (ECM) accumulation and in the pathogenesis of diabetic nephropathy (Zhu, Usui & Sharma, 2007). TGF-β1 is a member of a large family of polypeptides that exert their function through binding to specific receptors, including receptors type I, type II, type III (or betaglycan) and endoglin (Massagué, 1998).

Endoglin (ENG) mutations cause hereditary hemorrhagic telangiectasia type 1, also known as Osler-Weber-Rendu syndrome (McAllister et al., 1994). Soluble endoglin, a mutant form of endoglin, has recently been implicated in the pathogenesis of preeclampsia (Levine et al., 2006; Venkatesha et al., 2006), a condition associated with increased vascular permeability. Probably more relevant to this paper, endoglin/CD105, a 180 kDa homodimeric type I integral membrane protein, shows structural homology with betaglycan (Llorca, Trujillo, Blanco & Bernabeu, 2007) and functions as an auxiliary component of the TGF-β receptor complex (Letamendia et al., 1998). Endoglin binds TGF-β1, TGF-β3, activin-A, bone morphogenetic protein (BMP) -2 and BMP-7 in the presence of the signaling receptors type I and II (Barbara, Wrana & Letarte, 1999; Cheifetz et al., 1992; Guerrero-Esteo, Sanchez-Elsner, Letamendia & Bernabeu, 2002), and modulates TGF-β1-induced synthesis of ECM-related molecules including collagen, fibronectin, plasminogen activator inhibitor-1 (PAI-1) and lumican (Lastres et al., 1996). We have previously demonstrated that endoglin is up-regulated in kidneys of rodents with renal fibrosis induced by renal mass reduction (Rodriguez-Peña et al., 2001) or unilateral ureteral obstruction (Prieto et al., 2005; Rodriguez-Peña et al., 2002). Interestingly, endoglin was found to be expressed in human and rat mesangial cells and its expression was up-regulated by TGF-β1 (Diez-Marques et al., 2002; Rodriguez-Barbero, Obreo, Eleno, Rodriguez, Bernabeu & Lopez-Novoa, 2001). Endoglin-transfected mice fibroblasts show lower collagen, fibronectin and PAI-1 expression levels than control cells, either with or without treatment with TGF-β1 (Diez-Marques et al., 2002; Guerrero-Esteo et al., 1999).

These data suggest that the increased expression of endoglin in renal tissues could limit the extent of ECM accumulation, the pivotal abnormality in diabetic nephropathy (Zhu, Usui & Sharma, 2007). Given this background, we have studied endoglin expression in skin fibroblasts from patients with type 1 diabetes with and without diabetic nephropathy.

RESEARCH DESIGN AND METHODS

“Fast-track” and “slow-track” type 1 diabetic patients

Participants for this study were selected from a cohort of 125 type 1 diabetic patients with at least 8 years of diabetes duration and a wide range of kidney function, as described in detail elsewhere (Caramori et al., 2002). Briefly, and as selected for earlier gene expression studies (Huang et al., 2006), the 20 patients with the fastest rate of development of diabetic nephropathy lesions and proteinuria (“fast-track”) and the 20 patients with the slowest rate of development of diabetic nephropathy lesions and normoalbuminuria (“slow-track”) were selected for these studies. Research skin and kidney biopsies were obtained from each patient.

Type 1 diabetic sibling pairs

Twenty-one type 1 diabetic sibling pairs also had research renal biopsies and renal function studies in the University of Minnesota General Clinical Research Center (GCRC) as previously reported (Caramori et al., 2006). They all had at least 4 years of diabetes, serum creatinine <2.0 mg/dl, glomerular filtration rate (GFR) >40 ml/min/1.73 m^2 and no other kidney disease. Thirty-four (80%) had no clinical or laboratory evidence of renal disease. Glomerular structural parameters (Caramori et al., 2006; Fioretto et al., 1999) and gene

expression studies in of these sibling pairs have been previously reported (Caramori et al., 2006; Trevisan et al., 1999).

These studies were approved by the Committee for the Use of Human Subjects in Research of the University of Minnesota. Informed consent was obtained from all participants before each study.

Normal controls

Controls were 20 healthy volunteers without family history of diabetes.

Renal Function Studies

Blood pressure levels were assessed as described (Caramori et al., 2002). Hypertension was defined as blood pressure ≥130/85 mmHg or use of antihypertensive medication. HbA_{1c}, AER, serum and urinary creatinine were measured as previously reported (Caramori et al., 2002). Patients were classified as normoalbuminuric (AER ≥20 μg/min), microalbuminuric (AER 20–200 μg/min), or proteinuric (≥200 μg/min) depending on at least two of three 24 h AER measurements being in the same range. GFR was estimated by iothalamate or iohexol plasma clearance. The mean of three 24 h creatinine clearances at the General Clinical Research Center (GCRC) was taken as a GFR estimate in patients studied several years ago. These GCRC creatinine clearances are highly correlated with classic inulin clearances (Ellis, Steffes, Goetz, Sutherland & Mauer, 1986).

Renal structural studies

Percutaneous kidney biopsy tissue was processed for electron microscopy (EM) (Caramori et al., 2002) and measurements of glomerular basement membrane (GBM) width and fractional volume of glomeruli occupied by mesangium [Vv(Mes/glom)] were performed as detailed elsewhere (Caramori et al., 2002). The rate of development of diabetic nephropathy lesions was expressed as the estimated rate of mesangial expansion, using mesangial expansion score, defined as [measured Vv(Mes/glom) - mean normal Vv(Mes/glom)]/ diabetes duration in years × 100. Reference values for glomerular structural parameters were derived from 76 age-and sex-matched normal living kidney transplant donors (Caramori et al., 2002).

Cell Culture

A skin biopsy was performed with a 3-mm punch at the kidney biopsy site in diabetic patients and in a similar location in control subjects. The methods for cell culture and RNA isolation from these skin biopsies have been previously detailed (Caramori et al., 2006; Huang et al., 2002; Huang et al., 2004; Huang et al., 2006). Briefly, stored cells were thawed and cultured for three passages in DMEM (Gibco-Life Technologies, Grand Island, N.Y., USA) with 25 mmol/l glucose and 20% fetal calf serum (FCS; Hyclone Lab, Logan, Utah, USA). The 4^{th} passage skin fibroblasts were seeded in 75 cm^2 flasks at a density of 10^4 cells per cm^2 and grown in DMEM with 25 mmol/l glucose and 10% FCS for 24 h. After exposure to serum-deprived DMEM medium for 48 h, skin fibroblasts were cultured for an additional 36 h in the same medium supplemented with 10% FCS.

RNA isolation

Total RNA was isolated as described previously (Huang et al., 2006). RNA yield was measured by UV absorbency at 260 nm, and RNA integrity was evaluated using an Agilent 2100 Bioanalyzer and a RNA 6000 LabChip kit (Hewlett Packard, Palo Alto, California, USA). Degraded samples were replaced. Total RNA isolated from the skin fibroblasts of six

control subjects cultured in DMEM with 10% FCS for 72 h were pooled to generate the standard reference RNA for the QRT-PCR assay.

Microarray

Gene expression profiles were assessed using the Affimetrix HG-U133A GeneChips (Santa Clara, California, USA) accordingly to the manufacturer's protocol (Affymetrix Microarray Suite User's Guide, 2001), as previously described (Huang et al., 2006). Briefly, total RNA was isolated using Trizol Reagent followed by the RNeasy Mini Kit (Qiagen, Valencia, California, USA). Double-stranded cDNAs were prepared from equal amounts of purified total RNA, and biotinylated cRNA was synthesized from the purified cDNA. These cRNA were hybridized to the gene chip (containing ~ 18,400 transcripts and 14,500 genes) using the GeneChip Instrument System (Affymetrix). Normalization was used in the expression analysis settings to allow comparison between array experiments and groups. Microarray endoglin mRNA expression values are expressed as relative units.

Quantitative Real Time PCR

The methods for QRT-PCR have been previously detailed (Caramori et al., 2006; Huang et al., 2002; Huang et al., 2004). Primers and probes were designed by Primer Express version 1.5 (Applied Biosystems, Warrington, UK) to produce an amplicon spanning an intron. The sequence of the probe and the forward and reverse primers used for this study were: 5′-FAM-CCTCCGACTGGGCCAAGCCC-TAMRA-3′, 5′-AATGACCCCCAGAGCATCC-3′, 5′-ATGCAGAAGGACAGTGACCCC-3′, respectively. These probes were labeled with 6-FAM (6-carboxyfluorescein) at the 5′ end and TAMRA (6-carboxy-tetramethyl-rhodamine) at the 3′ end. The 50 μl QRT-PCR reaction contained 1× TaqMan Buffer A, 5.5 mmol/l $MgCl_2$, 300 μmol/l dNTP, 0.025U/μl AmpliTaq Gold DNA Polymerase, 0.25 U/μl MultiScribe Reverse Transcriptase, 200 nmol/l forward and reverse primers, 0.2 U/μl RNase inhibitor and 0.1 μg total RNA. After 30 min at 48 °C and 10 min at 95 °C, the amplification reaction was carried out through 40 cycles at 95 °C for 15 s and 58 °C for 60 s. Target mRNAs in unknown samples were quantitated as detailed previously (Caramori et al., 2006; Huang et al., 2002; Huang et al., 2004). Briefly, a set of dilutions of the reference total RNA was used to construct a standard curve and the expression levels of unknown samples were calculated by interpolating their threshold cycle into the regression equation for the standard curve. The relative value of target mRNA in 0.1 μg of unknown total RNA sample was expressed as fold changes based on the concentration of target mRNA in 0.1 μg of reference total RNA. Multiple quality control strategies for these mRNA measurements, as described earlier, were used (Huang et al., 2002).

Western blot analysis

Four subjects from each group ("fast-track" and "slow-track" patients and controls), representing a wide range of endoglin mRNA expression, were selected for the protein expression studies. Cells were lysed in buffer (140 mM NaCl, 10 mM EDTA, 10% glycerol, 1% NP-40, 20 mM Tris pH 7.5) containing protease inhibitors (Pepstatin 1μg/ml, Leupeptin 1μg/ml, Aprotinin 1μg/ml, PMSF 1mM, sodium ortovanadate) for 30 minutes in ice. Cell lysates were centrifuged at 12,000×*g* for 10 min at 4° C, and the supernatant collected. The protein content was determined by a commercially available variant of the Lowry method (Bio-Rad) using BSA as the standard. Fresh cell lysates were analyzed in 8% SDS–polyacrylamide gel.

Electrophoresis

Samples for endoglin detection were prepared in the Laemmli nonreducing buffer (final concentration: 125 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 1% bromophenol blue). For

endoglin detection, 25 μg of total protein was loaded. Gels were blotted onto PVDF membranes (Bio-Rad), and the membranes were blocked with 3% BSA Tris-buffered saline (TBS)-Tween (0.1%) overnight at 4° C. The membranes were then incubated with mouse anti-human endoglin monoclonal antibody TEA 1/58 (Luque et al., 1997) for 2 h at room temperature. Blots were then washed in TBS-Tween, followed by incubation with the secondary antibody, HRP-conjugated goat anti-mouse IgG (Bio-Rad), for 30 minutes. Blots were developed by chemiluminescence, using the ECL Western blotting system (Amersham-Biosciences) with films (Kodak BioMax Mr film). The bands were quantified using the Molecular Analyst software (Bio-Rad).

Statistical analyses

Summary data, including mean, standard deviation (SD), median, and range, were generated for all study variables. Results are presented as means ± SD, except for AER and GBM width that were not normally distributed and are presented as median and range. Microarray data were processed as previously reported by us (Huang et al., 2006). Analysis of variance (ANOVA) methods were used to compare continuous variables among “fast-track” patients, “slow-track” patients and control subjects. A Hochberg modification of the Bonferroni procedure (Hochberg, 1998) was used to perform multiple comparisons between groups; tests were performed only when the overall *F* test was significant. Comparisons for discrete variables were determined by χ^2 statistic. Pearson’s correlation coefficient (r) was used to determine the relationship between endoglin mRNA and endoglin protein expression. To determine the contribution of genetic factors on variations in SF endoglin mRNA expression levels, we constructed nuclear families from the sibling pair data and performed genetic variance component analyses using the SOLAR software package (Southwest Foundation for Biomedical Research, San Antonio, TX) (Almasy & Blangero, 1998) as previously described (Caramori et al., 2006). The relative contribution of genetic factors to each phenotype is then determined by the heritability (h^2), defined by the ratio of additive genetic variance to the residual phenotypic variance (after the removal of covariates). Thus, h^2 is presented as the percentage of the variability in mRNA expression levels (mean ± SE) that is explained by genetic factors. Statistical tests with $P<0.05$ were considered statistically significant. All the investigators except for the statisticians (G.B.R., M.E.M., S.S.R.) were blinded to the study group assignment for the gene expression studies.

RESULTS

“Fast-track” and “slow-track” type 1 diabetic patient

Demographic results—Demographic characteristics were similar among groups (Table 1). Glycated hemoglobin, systolic and diastolic blood pressures were higher and glomerular filtration rate was lower in “fast-track” compared to “slow-track” patients. AER, by design, was higher among “fast-track” *vs.* “slow-track” patients. Eighteen “fast-track” and none of the “slow-track” patients were receiving antihypertensive drugs ($p<0.001$). Eight “fast-track” patients were on angiotensin converting enzyme inhibitors (ACEi), 2 on angiotensin receptor blockers (ARB) and 8 were on antihypertensive medications other than ACEi or ARB; 2 “fast-track” patients were on no blood pressure medications. GBM width and Vv(Mes/glom) were greater in “fast-track” *vs.* “slow-track” patients (Table 1).

Microarray results—Endoglin mRNA expression levels were different among groups (ANOVA p=0.015). Control (1223.1 ± 422.9) and “fast-track” (1211.0 ± 274.9) groups had similar endoglin expression levels (p=0.76), whereas “slow-track” patients (1516.0 ± 349.9) had higher endoglin expression levels than controls (p=0.013) or “fast-track” patients (p=0.011). Use of ACEi or ARB had no effect on endoglin expression in the “fast-track” group (p=0.62).

QRT-PCR results—The microarray group differences were confirmed by QRT-PCR (ANOVA p=0.025). Thus, endoglin mRNA expression was higher in the "slow-track" group (2.07 ± 0.39) than in the "fast-track" (1.75 ± 0.27; p=0.009) or in the control (1.78 ± 0.50; p=0.021) group (Figure 1). Use of ACEi or ARB had no effect on endoglin expression in the "fast-track" group (p=0.65). There was a significant correlation (r=0.65; p=0.022) between endoglin mRNA levels measured by microarray and by QRT-PCR.

Protein expression results—Endoglin protein expression, detected in all samples studied, correlated directly with endoglin mRNA expression levels as measured by microarray (r=0.59; p=0.044) or by QRT-PCR (r=0.61; p=0.034).

Type 1 diabetic sibling pairs

Demographic characteristics of these sibling pairs have been previously reported (Caramori, et al., 2006). Briefly, these 42 diabetic patients (25 females) were 38.1 ± 7.9 years old, 15.9 ± 9.0 years old at diabetes onset and had diabetes for 21.5 ±10.6 years. HbA_{1c} at biopsy was 8.4 ± 1.2%. AER was 6.6 (1.0–860) μg/min; 34 were normoalbuminuric, 4 were microalbuminuric, 3 were proteinuric and 1 was unclassifiable. GFR ranged from 46 to 162 ml/min/1.73 m^2. Hypertension was present in 11 patients, 6 were on antihypertensive drugs (3 of them on ACEi or ARBs). The intraclass correlation between the sibling pairs was R0.19 (P<0.34) for endoglin mRNA expression levels. Using the variance component analyses model after adjustment for age, sex and diabetes duration, the estimated heritability for endoglin mRNA expression levels was h^2= 0.19 ± 0.46 (P=0.34).

CONCLUSIONS

Skin fibroblast behaviors in type 1 diabetes are related to diabetic nephropathy risk (Ceriello et al., 2000; Huang et al., 2002; Huang et al., 2004; Huang et al., 2006; Iori et al., 2000; Iori et al., 2003; Lurbe, Fioretto, Mauer, LaPointe & Batlle, 1996; Siczkowski, Davies, Sweeney, Kofoed-Enevoldsen & Ng, 1995; Tessari et al., 2007; Trevisan, Yip, Sarika, Li & Viberti, 1997). These differences in cellular behavior in cells grown under identical *in vitro* conditions may represent genetic predisposition to diabetic nephropathy, "memory" to the previous *in vivo* diabetic environment, or aspects of both phenomena. Genetic predisposition may also play a major role in determining diabetic nephropathy risk (Ewens, George, Sharma, Ziyadeh & Spielman, 2005; Krolewski, 1999; McKnight et al., 2006; Osterholm et al., 2007; Rich, 2006), but the role of cellular "memory" remains unresolved. Thus, the study of skin cells derived from type 1 diabetic patients at very high ("fast-track") or very low ("slow-track") risk of diabetic nephropathy and controls grown in identical conditions may provide a useful model for hypothesis generation.

Although, as noted above, studies from several different laboratories have shown skin fibroblast *in vitro* behavioral differences between patients at high *vs*. low risk of diabetic nephropathy and *vs*. normal controls, only our laboratory has provided evidence that the "slow-track" group of patients is also unique (Huang et al., 2002; Huang et al., 2004). In the present studies of endoglin gene expression, as well as in our previous studies of TGF-β binding protein (Huang et al., 2002) and GLUT-1 (Huang et al., 2004) gene expression, the "slow-track" group differed from both the "fast-track" group and controls, while the latter two groups did not differ. These consistent results may be directly related to the stringent selection criteria, especially for the "slow-track" subjects, which are based on both renal biopsy findings and renal function. Use of renal biopsy measurements to select patients at low diabetic nephropathy risk reduces experimental (and etiologic) heterogeneity, as patients with moderate to advanced diabetic glomerular lesions despite normal albumin excretion rates (Caramori et al., 2002; Caramori, Fioretto & Mauer, 2003; Fioretto, Steffes & Mauer,

1994; Torbjörnsdotter, Perrin, Jaremko & Berg, 2005) are not included in the "slow-track" group. The present study did not find evidence for significant heritability of endoglin gene expression levels in type 1 diabetic sibling pairs. Thus, differences between "slow-" and "fast-track" patients, which were present after several *in vitro* passages under identical conditions, could represent cell memory phenomena.

We have previously adopted the high throughput microarray approach to determine cultured skin fibroblast gene expression pathway differences between "fast-track" and "slow-track" type 1 diabetic patients (Huang et al., 2006). Another approach is to explore within pathways of interest, such as ECM dynamics. TGF-β as well as endoglin have been implicated in renal injury in mice, rats and humans (Prieto et al., 2005; Rodriguez-Peña et al., 2002; Rodriguez-Peña et al., 2004; Roy-Chaudhury, Simpson & Power, 1997; Zhu, Usui & Sharma, 2007). Thus, the finding of differential endoglin mRNA expression by microarray analyses led to our validation studies by QRT-PCR and to the study of the association of mRNA and protein expression levels in these cells. It is conceivable that drugs blocking the renin-angiotensin system could affect endoglin levels (Li, Chen & Mehta, 2001; Prieto et al., 2005). However, there were no differences in endoglin gene expression between the 10 "fast-track" subjects receiving and the 10 not receiving these drugs.

These studies could be of pathogenetic relevance. Considering that tissue damage ultimately results from an imbalance between injury and repair (or mediators and inhibitors of damage), it could be that the increased endoglin expression seen in damaged renal tissues (Prieto et al., 2005; Rodriguez-Peña et al., 2002; Rodriguez-Peña et al., 2004; Roy-Chaudhury, Simpson & Power, 1997) represents a protective mechanism that has been up-regulated in response to damage. Alternatively, and depending on other factors, endoglin could contribute to injury in some circumstances and be protective in others, as has been described for TGF-β itself (Border & Noble, 1993; Border & Ruoslahti 1992; Roberts, McCune & Sporn, 1992; Schnaper, Hayashida & Poncelet, 2002; Sharma & Ziyadeh, 1994). Two different forms of endoglin, S- and L-endoglin, with opposite effects on TGF-β regulation in myoblasts (Velasco et al., 2008; Pérez-Gómez at al., 2005) have been recently described. The antibody and the probe we used recognize both S- and L-endoglin, as they share all the extracellular sequence, and only differ in 7 residues of the intracellular tail, that is shorter in S- than in L-endoglin. However we cannot differentiate between S- and L-endoglin, the later is the most abundant isoform, being S-endoglin a minority form in all cell types studied so far, including fibroblasts (Rodriguez-Barbero and Lopez-Novoa, unpublished data). Thus, the results of the present manuscript most likely refer to L-endoglin.

There is an inverse relationship between endoglin expression and ECM synthesis in several cell culture models. An inhibitory role of endoglin on TGF-β1-induced PAI-1 and, to a lesser extent, fibronectin synthesis has been demonstrated in NCTC929 fibroblasts (Guerrero-Esteo et al., 1999). Endoglin expression also regulated basal and TGF-β1 induced ECM synthesis in L6E9 myoblasts (Rodriguez-Barbero, Obreo, Alvarez-Muñoz, Pandiella, Bernabeu & Lopez-Novoa, 2006). Over-expression of endoglin in L6E9 myoblasts led to a reduction in the TGF-β1-induced promoter activity of connective tissue growth factor (CTGF) (Obreo et al., 2004) as well as reduction of other TGF-β1 biological effects (Lastres et al., 1996; Llorca, Trujillo, Blanco & Bernabeu, 2007). We have also recently demonstrated that both endoglin protein and mRNA were up-regulated in the remnant kidneys of rats with glomerular sclerosis and hypertension induced by $5/6^{th}$ nephrectomy (Rodriguez-Peña et al., 2004) and in rats (Prieto et al., 2005) and mice (Rodriguez-Peña et al., 2002) with unilateral ureteral ligation. Endoglin also appears to be up-regulated in

human chronic renal disease (Roy-Chaudhury, Simpson & Power, 1997). In these situations, endoglin overexpression could be a cellular response for limiting injury.

Although the present studies document an association between *in vitro* endoglin expression and diabetic nephropathy, confirmation of the relevance of theses findings would require studies of renal tissues at various stages of diabetic renal disease.

In summary, the present report of increased endoglin mRNA expression levels in "slow-track" type 1 diabetic patients, and a direct relationship between endoglin mRNA expression and protein levels, is consistent with a protective role for endoglin in diabetic nephropathy. The elucidation of such mechanisms would require additional studies.

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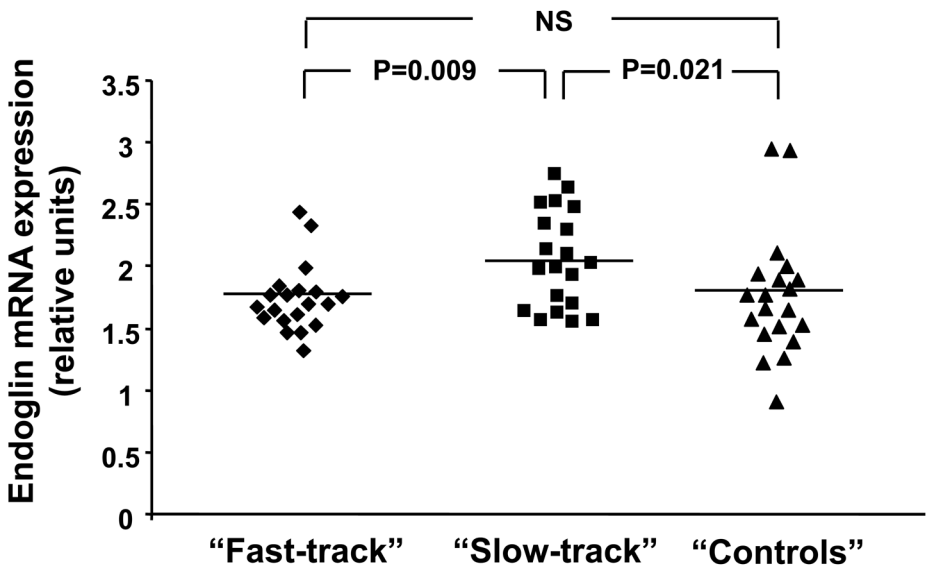

Figure 1.

Table 1

Demographic, clinical, and renal structural characteristics of type 1 diabetic patients and controls

	"Slow-track"	"Fast-track"	Controls	*P*
Male/Female	8/12	9/11	8/12	NS
Age (years)	36 ± 10	38 ± 8	41 ± 8	NS
Diabetes duration (years)	20 ± 8	24 ± 7		NS
HbA_{1C} (%)	8.4 ± 1.2	9.4 ± 1.5		0.027
Systolic blood pressure (mmHg)	117 ± 9	135 ± 11		<0.001
Diastolic blood pressure (mmHg)	69 ± 7	77 ± 7		<0.001
Hypertension (yes/no)	1/19	19/1		<0.001
Antihypertensive treatment (yes/no)	0/20	18/2		<0.001
Albumin excretion rate (μg/min)	5.3 (0.4–15.9)	599.6 (229–4630)		NA
GFR (ml/min per 1.73 m^2)	115 ± 13	72 ± 20		<0.001
GBM width (nm)	415 (329–737)	768 (515–1,249)		<0.001
Vv(Mes/glom)	0.20 ± 0.02	0.56 ± 0.10		<0.001
Mesangial expansion score	−0.012 ± 0.114	1.54 ± 0.490		NA

Data are the means ± SD, median (range), or number of subjects.

GBM: glomerular basement membrane; GFR: glomerular filtration rate; NA: not applicable, different by design; NS: not significant; Vv(Mes/glom): mesangial fractional volume.