High Glucose Level Unmasks a Genetic Predisposition to Enhanced Extracellular Matrix Production in Mesangial Cells from the Milan Normotensive Strain

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Abstract. A growing body of evidence indicates that the individual genetic background plays a role in the pathogenesis of diabetic glomerular disease by either favoring or protecting against injury produced by hyperglycemia. Two genetically related rat strains, the Milan normotensive strain (MNS) and the Milan hypertensive strain (MHS) display different susceptibilities to develop glomerulosclerosis with age. Glomerular sclerosing lesions occur in the MNS rats, which remain normotensive throughout their entire life-span, but not in the MHS rats, despite the presence of arterial hypertension. Previous studies have reported that extracellular matrix production and cell proliferation increased with donor-aging in mesangial cells isolated from MNS rats, but not in those from MHS rats, thus suggesting the existence of an inherited defect in the regulation of cell and matrix turnover, which translates into an abnormal response to growth-promoting stimuli favoring the development of glomerulosclerosis. In the study presented here, it was hypothesized that, in addition to donor-aging, other independent risk factors for the development of glomerular disease, such as metabolic injury by hyperglycemia, would be able to trigger and/or precipitate the occurrence of these changes in mesangial cells from the susceptible normotensive strain, but not in those from the protected hypertensive strain. To test this hypothesis, mesangial cells obtained from these rat strains (before the onset of either glomerulosclerosis or hypertension) were used to assess the effects of prolonged (4 wk) exposure to high (30 mmol/L) versus normal (5.5 mmol/L) glucose concentrations on extracellular matrix and cytokine production and cell proliferation. The accumulation and/or gene expression of the matrix components fibronectin, laminin, and collagen IV, and of the cytokines insulin-like growth factor-I (IGF-I) and transforming growth factor-β (TGF-β) did not change under normal glucose and increased progressively in response to high glucose in both MNS and MHS cells. These increases, with the exception of the increment in TGF-β gene expression, were significantly more pronounced in MNS cells than in MHS cells. In contrast, the proliferative response to serum was not affected by high glucose, but increased in MNS cells, and decreased, although not significantly, in MHS cells during the 4-wk period, thus mimicking the changes previously observed in these rat strains as a function of age. These results indicate that high glucose unmasks a genetic tendency to produce increasing amounts of extracellular matrix, not yet evident under normal glucose conditions, and suggest that a genetically determined propensity of mesangial cells to hyperrespond to chronic hyperglycemia may be implicated in the pathogenesis of diabetic glomerular disease. (J Am Soc Nephrol 8: 406–414, 1997)
the disease (11), and that KDDM can be prevented or retarded by strict metabolic control (12). However, epidemiological studies have indicated that prolonged exposure to poorly controlled diabetes is necessary but not sufficient to cause the development of KDDM. In fact, end-stage renal failure occurs only in a certain percentage of diabetic subjects, sometimes irrespective of metabolic control (13). Moreover, after a lag phase of 5 yr after IDDM onset, the cumulative incidence of proteinuria rapidly rises, peaks during the second decade of disease, and then declines (13), thus suggesting the existence of factors either favoring or protecting against the development of KDDM. The genetic nature of this susceptibility (or resistance) has been suggested by the demonstration of a familiar clustering of KDDM (14,15), which could not be explained by familiar sharing of an environmental risk factor. Genes coding for extracellular matrix components (ECM) components and genes involved in the regulation of blood pressure are believed to play a role in the pathogenesis of KDDM, although linkage studies have provided conflicting results (16).

Two genetically related rat strains derived from a common Wistar ancestor, the Milan normotensive strain (MNS) and the Milan hypertensive strain (MHS), display different susceptibilities to develop glomerulosclerosis (GS) with age (17). MNS rats develop an age-dependent form of GS (18–20) similar to abilities to develop embolusclerosis (GS) with age (17). MNS Milan hypertensive strain (MHS), display different susceptibilities to develop chronic kidney disease, although linkage has been suggested by the demonstration of a familiar clustering of KDDM (14,15), which could not be explained by familial sharing of an environmental risk factor. Genes coding for extracellular matrix components (ECM) components and genes involved in the regulation of blood pressure are believed to play a role in the pathogenesis of KDDM, although linkage studies have provided conflicting results (16).

In previous studies, one of us has reported that serum-stimulated cell proliferation and ECM production increased with donor aging in mesangial cells isolated from MNS rats, thus suggesting the existence of an inherited defect in the regulation of cell and matrix turnover, which translates into an abnormal response to growth-promoting stimuli favoring the development of GS (26). Conversely, mesangial cells from MHS rats showed an age-associated decline in proliferative activity and unchanged matrix synthesis, which could reflect a process of normal cell aging, possibly protecting this strain from the development of glomerular disease despite the presence of AH (26).

Based on these previous observations, superimposing the diabetic milieu to the (1) genetic susceptibility to GS associated with normal blood pressure, in mesangial cells from MNS rats, or (2) protection from GS associated with genetic AH, in mesangial cells from MHS rats, may represent a model for investigating the interaction between metabolic and genetic factors with respect to changes in mesangial ECM deposition and cell proliferation underlying the expansion of mesangium. We hypothesized that an independent risk factor for the development of GS, such as metabolic injury by hyperglycemia, could be able to trigger and/or precipitate the occurrence of these changes in the susceptible normotensive strain, just as age normally does in these animals, but not in the protected hypertensive strain.

To test this hypothesis, we assessed the effects of high glucose levels on ECM and cytokine production and cell proliferation in cultured mesangial cells isolated from MNS and MHS rats before the development of either GS or AH.

**Materials and Methods**

**Experimental Design**

Mesangial cells from MNS and MHS rats were cultured for 4 wk (through four passages) under (1) normal (5.5 mmol/L glucose (NG)), (2) high (30 mmol/L glucose (HG)), and (3) 24.5 mmol/L mannitol + NG (to match osmolality of HG medium) levels.

At each passage, the following parameters were assessed: (1) the medium levels of the ECM components fibronectin (FN), laminin (LAM), and Type IV collagen (C-IV), IGF-I, and IGF binding proteins (IGFBP); (2) DNA levels (to which the above parameters were normalized); (3) transcripts for FN, C-IV, IGF-I, and TGF-β; and (4) cell proliferation.

**Isolation, Characterization, and Culture of Mesangial Cells**

Glomeruli were isolated from kidneys obtained from four MNS or MHS rats of 1 month of age (before the onset of either GS or AH) using standard sieving techniques, as previously reported (6,10). The resulting mesangial cells were characterized according to immunological, histological, and functional criteria, as described in previous publications (6,10).

Mesangial cells between the third and the tenth passage were cultured in Dulbecco's modified Eagle medium (DMEM; Sigma, St. Louis, MO) supplemented with 17% fetal bovine serum (FBS), 2 mmol/L L-glutamine, antibiotics (all obtained from Flow Laboratories, Irvine, Scotland, UK), but without insulin, at 37°C in a 95% air/5% CO₂ humidified atmosphere (6,10), under the experimental conditions indicated above.

**Assessment of ECM Accumulation**

Mesangial cells were plated onto 35-mm multiwell dishes (Falcon). At subconfluence, cells were exposed for 24 h to serum-free medium containing 0.3 mmol/L ascorbic acid (Sigma) and 0.4 mmol/L β-aminopropionitrile (Sigma) facilitating matrix protein secretion and inhibiting ECM assembly, respectively; under these conditions, most of the ECM proteins synthesized are found in the medium with no change in the overall production rate (6,10).

The medium levels of the ECM components FN, LAM, and C-IV were quantified by an ELISA, as previously described (6,10). Rabbit polyclonal antibodies produced against rat FN (Calbiochem), mouse LAM (Bi chain), and mouse C-IV (NC1 globular domain) were used at 1:72,000, 1:2,500, and 1:2,500 dilutions, respectively. All antibodies were highly specific and reacted or crossreacted with rat ECM proteins (6,10). Intra-assay and interassay variations ranged from 5% to 10% for all assays. Detection limits were 0.1 ng/well for FN, 0.25 ng/well for LAM, and 0.25 ng/well for C-IV.

**Assessment of IGF-I Production**

Cells were plated onto 35-mm multiwell culture dishes (Falcon). At subconfluence, cells were maintained for 24 h in serum-free medium, then media were collected, lyophilized, and reconstituted with water to obtain 5- to 10× concentrated samples.

IGF-I concentration in media was measured by the use of a RIA kit (Nichols Institute, San Juan Capistrano, CA), after ethanol (87.5%) - 2 N HCl (12.5%) extraction of samples followed by neutralization with 0.855 M Tris-base (27). The RIA procedure was slightly modi-
Assessment of IGFBP Production

Cells were cultured in 35- or 100-mm multiwell culture dishes (Falcon) and, at subconfluence, were maintained for 24 h in serum-free medium. Media were then collected in tubes pretreated with DMEM containing 0.1% BSA at 37°C for 3 h under agitation (to decrease protein background), lyophilized, and reconstituted with water to obtain 10 to 20× concentrated samples.

IGFBP production was evaluated by Western ligand blot (29). Medium samples were subjected to nonreducing 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then blotted onto 45 μm nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) by a transblotting apparatus (Bio-Rad, Richmond, CA). The membranes were prewashed with Tris-buffer (150 mmol/L NaCl and 10 mmol/L Tris-HCl, pH 7.4) containing 0.5 mg/mL sodium azide and 3% NP40, incubated for 4 h in Tris-buffer with 1% BSA, for 10 min in Tris-buffer plus 0.1% Tween 20, and for 2 h at room temperature in Tris-buffer containing 1% BSA, 0.1% Tween 20, and 2 × 10⁶ cpm of 125I-IGF-II (200 Ci/mmol, Amersham), washed three times at 4°C with Tris-buffer, dried, and exposed at -70°C for 3 days to X-Omat RP autoradiography films (Eastman Kodak Co., Rochester, NY) with the aid of intensifying screens. Quantification of IGFBP was performed by scanning densitometry using a GS-670 Imaging Densitometer (Bio-Rad Laboratories, Hercules, CA).

Assessment of DNA Levels

Results of ECM and IGF-I measurements were normalized to DNA content of monolayers extracted with 0.5 N NaOH and sonicated (6,10).

DNA levels were measured fluorimetrically after reaction of cell extracts with 0.6 mol/L 4,6-diamidino-2-phenylindole (Sigma) (30). DNA measurements were performed in a Perkin-Elmer LS-50 fluorimeter (Perkin-Elmer Co., Norwalk, CT) at 352 nm excitation and 454 nm emission, using 10-nm slits.

Assessment of ECM and Growth-Factor Gene Expression

Subconfluent cells, grown in 100-mm Petri dishes (Falcon), were maintained in serum-free medium for 24 h, then total RNA was extracted by the guanidine thiocyanate-phenol-chloroform method (31) using the RNAfast-II kit (Molecular Systems, San Diego, CA).

The levels of transcripts for FN, C-IV, IGF-I, and TGF-β1 were measured by Northern Blot analysis, as previously described (10). Twenty to 40 μg of total RNA per lane were electrophoresed under denaturing conditions in 1.2% agarose (Life Technologies, Gaithersburg, MD) gel, transferred onto Hybond-N nylon blotting membranes (Amersham) and ultraviolet-fixed in Spectrolinker XL-1000 UV (Spectronics Corporation, Westbury, NY). Filters were prehybridized for 6 h at 42°C in 50% formamide (Fluka Chemie AG), 5 × SSPE, 5 × Denhardt’s solution, 100 μg/mL of salmon sperm DNA (Sigma), and 0.1% SDS and then hybridized overnight at 42°C in the same solution containing 25 ng of cDNA labeled by the random-primer method (32) using the Random Primer Labeling Kit Prime-IT™ II (Stratagene, La Jolla, CA) and 50 μCi of [α-32P]dCTP (3000 Ci/mmol, Amersham) as precursor. The 500-base pair (bp) EcoRI rat FN cDNA (33), the 830-bp PstI-Aval rat α1 C-IV cDNA (34), the 505-bp Sau3A-NlaIV rat IGF-I cDNA (35), the 550-bp Sma I human TGF-β1 cDNA (36), and the 2200-bp Bam HI human β-actin cDNA (37) were used as probes. Filters were sequentially washed for 15 min at 65°C with standard saline citrate (SSC) 2× (twice), SSC 2× + 0.1% SDS, and SSC 0.1× and then exposed to Hyperfilm-MP autoradiography films (Amersham) with the aid of intensifying screens at -70°C for 1 to 7 days. ECM and cytokine mRNA levels were quantified by scanning densitometry. To account for differences in sample loading, results were normalized to the β-actin signal.

Assessment of Cell Proliferation

Cell proliferation was determined as both DNA synthesis by 3H-thymidine incorporation (6) and cell number by crystal violet binding to cell nuclei (38) in cells grown in 16-mm multiwell culture dishes (Falcon). At confluence, cells were maintained for 72 h in quiescence and, subsequently, for 24 h in the presence of 0.5% or 17% FBS.

In the 3H-thymidine incorporation experiments, cells were then pulsed for 4 h with 1 μCi/mL of methyl-3H-thymidine (25 Ci/mmol, Amersham). Subsequently, media were aspirated off and cells were washed with phosphate-buffered saline (PBS), solubilized for 2 h at room temperature in 0.1% SDS plus 0.1% BSA as a carrier protein, and precipitated with 100% trichloroacetic acid (TCA; Sigma). After incubation for 30 min at 4°C, the TCA-precipitable material was pelleted by centrifugation, redissolved in 0.1% SDS, incubated overnight at room temperature, and processed for liquid scintillation counting. Results of 3H-thymidine uptake were normalized per protein content of each well, as measured by the Bradford method (39) on aliquots of samples using BSA as standard.

In the crystal violet binding experiments, medium was removed and monolayers were fixed in 1% glutaraldehyde (Sigma) dissolved in Hank’s balanced salt solution (HBSS; Sigma) for 15 min at room temperature. Glutaraldehyde was then removed, fixed cells were treated with 0.1% crystal violet (Sigma) for 30 min at room temperature, and dishes were gently rinsed to remove unbound dye, and air-dried. Subsequently, crystal violet pigments in the cell nuclei were dissolved in 0.1% aqueous Triton X-100 (Sigma) for 4 h at room temperature under slow-speed shaking. Finally, aliquots of each sample (in duplicate) were transferred into 96-well microplates (Nunc, Roskilde, Denmark) and the absorbance at 600 nm was measured in a Titertek Multiscan MC (Flow Laboratories).

Statistical Analyses

Results are expressed as mean ± SD or as mean ± SD of percent variations versus the corresponding controls. The contribution of donor-rat strain (MNS versus MHS) and glucose concentration in the culture medium (NG versus HG) as well as of the interaction between these two variables at each time point was evaluated by two-way analysis of variance (ANOVA). The effects of time in culture in each experimental group was analyzed by one-way ANOVA followed by Tukey studentized range method for comparison among the different time points. All statistical tests were performed on raw data.

Results

Extracellular Matrix

Under NG conditions, ECM accumulation did not change during the 4-wk period in cells from both MNS and MHS rats, and did not differ between the two cell lines (Figure 1). In response to HG, ECM accumulation increased progressively with time in both MNS cells (P < 0.001) and MHS cells (P < 0.001 for FN and LAM, and P < 0.005 for C-IV). At Week 4, FN, LAM and C-IV accumulations were increased versus NG conditions.
FIGURE 1. Medium content of the ECM components FN, LAM, and C-IV, as assessed by ELISA, in mesangial cells from MNS and MHS rats during a 4-wk culture under HG versus NG conditions. (●●●) MNS-NG; (■■■) MNS-HG; (*--*) MHS-NG; (△△△) MHS-HG (seven to 11 experiments per each condition/time).

by 70%, 74%, and 76%, respectively, in MNS cells, and by 48%, 43%, and 52%, respectively, in MHS cells (Figure 1). The effect of HG was statistically significant starting at Week 1 for FN (P < 0.01, and P < 0.001 thereafter) and at Week 2 for LAM and C-IV (P < 0.001), whereas the effect of donor-rat strain was significant at Weeks 3 (P < 0.05) and 4 (P < 0.001) for FN and at Week 4 for LAM and C-IV (P < 0.05). The interaction between glucose concentration and rat strain achieved the statistical significance at Week 2 for FN (P < 0.01, and P < 0.001 thereafter) and at Week 4 for LAM and C-IV (P < 0.05). Likewise, transcripts for FN and C-IV were unchanged in cells cultured under NG and were progressively increased by HG in both cell lines during the 4-wk period (P < 0.001) (Figure 2). HG-induced increases in FN mRNA levels and, to a lesser extent, in C-IV mRNA levels were more pronounced in MNS cells compared with MHS cells, as evidenced by the significant contribution of strain and the combination of strain plus glucose concentration (P < 0.001 for FN at Weeks 3 and 4 and P < 0.05 for C-IV at Week 4). Iso-osmolar mannitol did not affect ECM accumulation and gene expression (not shown).

Cytokines

IGF system. IGF-I peptide (Figure 3) and mRNA (Figure 4) levels were unchanged in cells cultured under NG conditions, with no difference between MNS and MHS cells, as evidenced by the significant contribution of strain and the combination of strain plus glucose concentration (P < 0.001 for FN at Weeks 3 and 4 and P < 0.05 for C-IV at Week 4). Iso-osmolar mannitol did not affect ECM accumulation and gene expression (not shown).

Cell Proliferation

During the 4 wk of observation, the serum-stimulated increment (over serum-free conditions) of both 3H-thymidine incorporation and crystal violet binding to cell nuclei under NG conditions increased by 14% and 15%, respectively, in MNS cells, and decreased by 6% and 7%, respectively, in MHS cells, by 0.001). The increases induced by HG were more pronounced in MNS cells compared with MHS cells, with the effect of strain achieving the statistical significance at Week 4 (P < 0.001) and that of the interaction between strain and glucose concentration at Weeks 3 and 4 (P < 0.01) for both IGF-I medium content and mRNA levels. As previously reported, the Western blot analysis of IGFBP showed several bands with molecular weights ranging from 40 to 18 kd. The IGFBP production pattern did not change significantly in response to HG, except at Week 4, when a significant decrease was detected in all IGFBP bands (P < 0.001). No difference was observed between the two cell lines (Figure 4).

TGF-β. As for IGF-I, TGF-β gene expression did not change significantly with time under NG conditions and progressively increased under HG (P < 0.001), but not under iso-osmolar mannitol; however, at variance with IGF-I peptide and mRNA levels, the increments in TGF-β mRNA expression induced by HG were not different between the two cell lines (Figure 4). In fact, although the effect of glucose concentrations was statistically significant at Weeks 2 through 4 (P < 0.001), no effect of strain and the combination of the two variables was detected by two-way ANOVA.
but only the increment in $^3$H-thymidine uptake achieved the statistical significance ($P < 0.05$). Despite the different trends in the proliferative response to serum observed in the two cell lines, no significant effect of donor-rat strain was detected. Likewise, these indexes of cell proliferation were not affected by HG or iso-osmolar mannitol in both cell lines (not shown).

### Discussion

Mesangial cells from animals genetically predisposed to GS (MNS) or AH (MHS) did not change matrix and growth factor synthesis during a 4-wk culture under NG conditions, but responded to prolonged exposure to HG with progressively increased ECM accumulation and gene expression, IGF-I production, and IGF-I and TGF-β mRNA levels. The increases in FN, LAM, and C-IV accumulation and gene expression and IGF-I peptide and mRNA levels were significantly more pronounced in cells from GS-susceptible MNS rats, than in cells from GS-protected MHS rats, thereby supporting the concept that the extent of glomerular injury induced by hyperglycemia is modulated by the individual genetic background. At variance with ECM and growth factor production, the proliferative response to serum did not change in response to HG, but increased in MNS cells and decreased, although not significantly, in MHS cells throughout the observation period under NG conditions, thus mimicking the changes previously observed in these rat strains as a function of age.

The finding that ECM (and growth factor) synthesis did not change significantly in MNS cells from 1-month-old rats when cultured under NG conditions for 4 wk, both in absolute terms and relatively to cells from age-matched MHS rats, indicates that the inherited tendency to ECM overproduction becomes evident at a later donor age, as previously reported in cells from 8-month-old animals (after the development of GS) (26). In keeping with our earlier report (26), a trend toward in-
increased cell proliferation, in MNS monolayers, and decreased cell proliferation, in MHS monolayers, when cells were cultured under NG conditions, was already present at a donor age of 1 month. Taken together, these donor age-dependent changes in both proliferative and secretory activity observed in MNS and MHS cells under NG conditions, provide further support to the hypothesis that increased mesangial cell proliferation may represent an early pathogenetic phase of the glomerular sclerosing process (40-42).

The changes in ECM and cytokine production observed in both cell lines in response to HG mimicked those reported in our previous studies in normal (Sprague-Dawley) rat (6) and human (10) mesangial cells cultured under elevated glucose concentrations showing a progressive upregulation of the ECM components FN, LAM, and C-IV and of the cytokines IGF-I and TGF-β. At variance with these earlier works, the increments in IGF-I peptide and mRNA levels induced by HG were not associated with marked reductions in IGFBP production, although a trend toward a decrease in IGFBP binding capacity of media was observed, thus suggesting an increased IGF-I action (43). Both IGF-I and TGF-β may mediate the effects of hyperglycemia on matrix turnover by virtue of their ability to promote ECM protein synthesis (44). In fact, several experimental observations have indicated that these cytokines may play a role in diabetic glomerulopathy. In particular, the findings that mice transgenic for GH or GHRH develop glomerular lesions indistinguishable from those of diabetic animals (45) and that octreotide is capable of preventing albuminuria and sclerosis in rats with experimental diabetes (46) have prompted the proposal that the GH axis is involved in diabetic GS, possibly via local IGF-I production. Likewise, the ability of an anti-TGF-β antibody to prevent the increase in collagen gene expression and protein synthesis induced by HG in murine mesangial cells (47) has highlighted the importance of TGF-β upregulation, which seems to be dependent on the local activation of angiotsin II (48). Angiotsin II could also exert direct effects on glomerular growth and promote the synthesis and/or release of other cytokines, including basic fibroblast growth factor and platelet-derived growth factor (48). The parallelism between changes in IGF-I and TGF-β synthesis and changes in ECM production shown in this study, as well as in previous works, further supports (although it does not prove) the hypothesis that an upregulation of these cytokines induced by hyperglycemia may play a role in modulating the enhanced ECM accumulation leading to mesangial expansion. The trend of alterations in matrix and cytokine production observed in both cell lines suggests that the effects of HG are mediated by a time-dependent mechanism, such as nonenzymatic glycation with formation of advanced glycation endproducts, which were shown to accumulate at the intracellular level within 1 wk of culture under HG conditions (49).

The observation that DNA synthesis and cell number were not affected by HG in both cell lines is also in keeping with previous findings obtained in cells from normal Sprague-Dawley rats (6) and may be dependent on the concomitant upregulation of IGF-I and TGF-β, with opposing actions on cell replication (44). This finding supports the hypothesis that the expansion of mesangium induced by chronic hyperglycemia, at variance with other forms of glomerular sclerosing injury, involves predominantly the ECM, with the cell component playing only a minor role, if any (3).

The fact that the HG-induced increases in ECM accumulation and gene expression observed in mesangial cells from MNS rats were more pronounced than those observed in cells from MHS rats indicates that the genetic tendency to abnormal matrix deposition, not yet evident under NG conditions at this age, can be unmasked and/or accelerated by an independent risk factor, such as HG. The quantitative differences observed in the two cell lines after exposure to HG could be the result of an exaggerated response of MNS versus MHS cells to cytokines such as IGF-I and TGF-β, which were produced in increasing amounts by both cell types when cultured in HG. In addition, the more marked increase in IGF-I production induced by HG in MNS versus MHS cells could have participated in the modulation of the accelerated ECM accumulation observed in MNS versus MHS cells. The observation that the difference in medium accumulation of C-IV between MNS and MHS cells grown in HG was more marked than the difference in the level of transcripts for this ECM component suggests...
that the abnormal matrix deposition observed in MNS cells compared with MHS cells is attributable to both enhanced synthesis and impaired degradation of C-IV (50).

The finding that mesangial cells from MHS rats also increased matrix and cytokine production in response to HG, although significantly less markedly than MNS cells, provides further support to the hypothesis that HG per se is capable of upregulating ECM deposition via independent pathways possibly involving an increased synthesis and/or action of IGF-I and TGF-β.

When extrapolated to the in vivo situation, this finding suggests that diabetes may trigger an exaggerated growth response in glomeruli from predisposed individuals which ultimately develop ESRD, whereas it produces only “background” changes in subjects not carrying this unfavorable and possibly heterogeneous predisposition. These genetically determined quantitative differences in the glomerular growth response to hyperglycemia would therefore determine, together with the degree and duration of metabolic derangement, the observed variability in the extent of renal involvement among diabetic individuals (13).

In conclusion, these results indicate that hyperglycemia is
capable of unmasking an unrelated genetic tendency to abnormal ECM production, such as that underlying the GS occurring in MNS rats with increasing age, thus suggesting that a genetically determined propensity of mesangial cells to hyperrespond to chronic hyperglycemia may be implicated in the pathogenesis of diabetic glomerular disease.

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