Glomerulotubular Junction Abnormalities Are Associated with Proteinuria in Type 1 Diabetes

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Glomerulotubular junction abnormalities, frequent in proteinuric patients with type 1 diabetes, may contribute to the progressive GFR loss in overt diabetic nephropathy. Glomerulotubular junction abnormalities were examined in patients who have type 1 diabetes with a wide range of albumin excretion rates (AER). Renal biopsies from five normoalbuminuric patients, five microalbuminuric patients, six proteinuric patients, and five control subjects were studied by light and electron microscopy. Light microscopy specimens were serially sectioned to find and classify glomerulotubular junctions. Glomerular structural parameters were estimated using stereologic methods. Glomerulotubular junction abnormalities were found in 2% of glomeruli from control and normoalbuminuric patients and in 4% of glomeruli from microalbuminuric patients. In contrast, 71% of glomeruli from proteinuric patients had glomerulotubular junction abnormalities, including five (8%) atubular glomeruli. Electron microscopy findings were typical of diabetic nephropathy. Piece-wise linear regression models with glomerular, glomerulotubular junction, and interstitial parameters as independent variables provided greater GFR (92%; $P < 0.005$) and AER (95%; $P < 0.01$) prediction than multiple regression models (81% for GFR and 72% for AER). Thus, glomerular adhesions and glomerulotubular junction abnormalities help to explain the progressive GFR loss that is associated with onset of proteinuria in type 1 diabetes. Moreover, nonlinear models provide better fit for structural–functional relationships in patients with type 1 diabetes.


Diabetic nephropathy (DN) remains the leading cause of ESRD in the United States (1). Albumin excretion rate (AER) is the best predictor of nephropathy risk in patients with diabetes (2). Studies of structural–functional relationships have improved the understanding of the pathophysiology of DN. However, much of the variability in renal function observed in patients with type 1 diabetes is not explained by present models. Mesangial fractional volume [Vv(Mes/glom)] and glomerular basement membrane (GBM) width explained 59% of the variability in AER in a group of 125 patients who had type 1 diabetes with wide ranges of renal structure and function and underwent renal research biopsies. Surface density of peripheral GBM [Sv(PGBM/glom)], AER, and gender, together, explained only 33% of the variability in GFR among these patients (3). In fact, whereas GFR is normal or increased in most normoalbuminuric (NA) and microalbuminuric (MA) patients with type 1 diabetes and decreased in most proteinuric patients, there is substantial overlap in these structural parameters among these three groups (3). These findings suggest that other structural parameters also may be involved in the process of DN development and progression. Recently, we showed that both atubular glomeruli (AG) and glomerulotubular junction (GTJ) abnormalities (GTJA), defined as atrophic proximal tubule at the GTJ, are frequent in proteinuric patients with type 1 diabetes (4). These abnormalities were closely associated with tip lesions (glomerular tuft to Bowman’s capsule adhesions [TBCA] at GTJ area), a finding reported in a variety of renal diseases with clinical proteinuria (5–7). This study also found that, along with the classical diabetic glomerulopathy lesions, these GTJA, together with tubulointerstitial parameters, predicted most of the variability in GFR among these proteinuric patients (4). However, because NA and MA patients were not included, the relationship of GTJA and AG to AER could not be assessed in this study. Our study explores the frequency of tip lesions, GTJA, and AG over a wide range of AER; investigates the frequency of TBCA in these patients; and presents improved statistical models for the understanding of structural–functional relationships in patients with type 1 diabetes.

Materials and Methods

Patients

Five NA, five MA, and six proteinuric patients with type 1 diabetes and five normal kidney donors (controls) were included in this study. The groups were matched for age, gender, and diabetes age at onset and duration in groups with type 1 diabetes (Table 1). Some of the data from three of the proteinuric patients with type 1 diabetes and one of the control subjects were published previously (4). All studies were performed with permission of the Committee on the Use of Human Subjects in Research at the University of Minnesota and after informed consent was obtained.

Clinical Studies

Patients with type 1 diabetes were admitted to the General Clinical Research Center (GCRC) at the University of Minnesota for renal...
opsy tissues were embedded in paraffin, serially sectioned at 5-μm thickness, and stained with periodic acid-Schiff. Eight to 13 complete nonsclerosed glomeruli with intact Bowman’s capsules that were observable through serial sections were acquired using the disector method (12), which allows unbiased sampling of glomeruli regardless of their size. Glomeruli with sclerosis of 75% of the tufts on serial sections were enumerated for structural–functional relationship models (13). Relative GV was defined as volume of a given glomerulus divided by the mean GV from the same group and scored on the basis of the severity of tubular atrophy as previously described (4). Patients were classified into three groups on the basis of AER levels in at least two of three consecutive urine samples: NA, AER < 20 μg/min; MA, AER 20 to 200 μg/min; and proteinuric, AER > 200 μg/min. GFR was estimated from the clearance of iothalamate or iohexol, both shown to be interchangeable with the clearance of inulin (9). Values of GFR were not corrected for body surface area in the exploratory models (10).

Light Microscopy Studies. Zenker-fixed percutaneous renal biopsy tissues were embedded in paraffin, serially sectioned at 5-μm thickness, and stained with periodic acid-Schiff. Eight to 13 complete nonsclerosed glomeruli with intact Bowman’s capsules that were observable through serial sections were acquired using the disector method (12), which allows unbiased sampling of glomeruli regardless of their size. Glomeruli with sclerosis of >75% of the tufts on serial sections were enumerated for structural–functional relationship models but not considered for stereologic measurements. Glomerular tuft and corpuscle (tuft plus Bowman’s space) volumes were measured using the Cavalieri principle on digital images of serial profiles of glomeruli at ×300, and tuft to corpuscle ratio and mean glomerular volume (GV) per biopsy were calculated (13). Relative GV was defined as volume of an individual glomerulus divided by the mean GV from the same biopsy (4). Index of arteriolar hyalinosis was determined on serial sections semiquantitatively as detailed elsewhere (14). Images of renal cortex acquired by systematic uniform random sampling were used to estimate the fractional volumes of interstitium [Vv(PT/cortex)], proximal [Vv(Pth/cortex)] and distal [Vv(DT/cortex)] tubules per cortex, and atrophic tubules per total tubules [Vv(AT/TT)] using point counting (3). Tubular profiles with short, flat, or lost epithelial cells and with thick, wrinkled, and/or duplicated tubular basement membranes (TBM) were classified as atrophic. Serial profiles of glomeruli were examined carefully to identify GTJ and TBCA. GTJA were classified and scored on the basis of the severity of tubular atrophy as previously defined (4): Short atrophic tubules (SAT), in which the length of the atrophic segment is a few cells (Figure 1A); long atrophic tubules (LAT), in which the atrophic segment is longer than a few cells (Figure 1B); atrophic tubules with no observable glomerular opening (ATNO),

**Table 1. Demographic and clinical data of patients and control subjects**

<table>
<thead>
<tr>
<th>Group</th>
<th>F/M</th>
<th>Age (yr)</th>
<th>D (yr)</th>
<th>HT (Yes/No)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>HbA1c (%)</th>
<th>AERb (μg/min)</th>
<th>GFR (ml/min per 1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2/3</td>
<td>30 ± 8</td>
<td>—</td>
<td>0/5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NA</td>
<td>2/3</td>
<td>29 ± 6</td>
<td>14 ± 5</td>
<td>0/5</td>
<td>119 ± 7</td>
<td>65 ± 9</td>
<td>8.1 ± 2.2</td>
<td>6.5 ± 3.0</td>
<td>112 ± 5</td>
</tr>
<tr>
<td>MA</td>
<td>3/2</td>
<td>26 ± 10</td>
<td>14 ± 5</td>
<td>1/4</td>
<td>120 ± 13</td>
<td>73 ± 3</td>
<td>10.7 ± 2.1</td>
<td>56 ± 44</td>
<td>113 ± 15</td>
</tr>
<tr>
<td>P</td>
<td>3/3</td>
<td>34 ± 8</td>
<td>17 ± 3</td>
<td>6/0</td>
<td>137 ± 14c,d</td>
<td>82 ± 7d,e</td>
<td>9.4 ± 1.2</td>
<td>441 ± 248</td>
<td>80 ± 29g,h,i</td>
</tr>
</tbody>
</table>

aData are mean ± SD and range. AER, albumin excretion rate; C, control subjects; D, diabetes duration; DBP, diastolic BP; F, female; HbA1c, hemoglobin A1c; HT, hypertension; M, male; MA, microalbuminuric; NA, normoalbuminuric; P, proteinuric; SBP, systolic BP.

bDifferent in groups by design.

P < 0.03 versus NA.

P < 0.05 versus MA.

P < 0.005 versus NA.

P < 0.05 versus NA.

Figure 1. Glomerulotubular junction (GTJ) abnormalities (GTJA). (A) Glomerulus attached to a short atrophic tubule (SAT). The arrow points to the atrophic segment. (B) Glomerulus attached to a long atrophic tubule (LAT). The arrow points to the atrophic segment and tuft adhesion. (C) Glomerulus attached to an atrophic tubule with no observable opening (ATNO) and a tip lesion (arrow). (D) Atubular glomerulus (AG). *Tubular remnants that possibly belonged to the AG.
in which no connection between the Bowman’s space and the tubular lumen is discernible (Figure 1C); and AG, in which no tubular attachment to the glomerulus is found (Figure 1D) (4). SAT were scored +1, LAT as +2, ATNO as +3, and AG as +4, and the index of junctional atrophy (IJA) was calculated as the average of scores among glomeruli (4).

Electron Microscopy Studies. Tissues were prepared for electron microscopy as described previously (15). Random ultrathin sections through glomeruli were stained and examined as reported previously (15). Three nonsclerosed intact glomeruli (except in two cases in which only two suitable glomeruli were available) were used for the following estimates. Vv(Mes/glom), fractional volume of mesangial cells, [Vv(MC/glom)], and mesangial matrix [Vv(MM/glom)] per glomerulus were estimated using point counting (15). Sv(PGBM/glom) was estimated using an line grid (16) at ×3900 magnification. Total filtration surface (TFS) was calculated as product of Sv(PGBM/glom) and glomerular volume. GBM width was estimated at ×11,700 using the orthogonal intercept method (17).

Statistical Analyses
Statistica 5.0 (StatSoft, Inc., Tulsa, OK) was used for statistical analyses. AER data were logarithmically transformed. Mean group values were compared by ANOVA. Homogeneity of variances was evaluated by the Levene test. Multiple regression (forward stepwise) and piecewise linear regression analyses (PLRA) were performed to model structural-functional relationships in patients with type 1 diabetes. The quasi-Newton method of estimation was used to minimize loss function (maximum likelihood), and the software was allowed to estimate one possible breakpoint for each model. All data are expressed as mean ± SD unless specified otherwise. P < 0.05 was considered statistically significant.

Results
Demographic and Clinical Features
Age in all groups and age at onset and duration of type 1 diabetes in the groups with diabetes were similar by design (Table 1). Hypertension was almost entirely confined to proteinuric patients who were taking angiotensin-converting enzyme inhibitors. There were no group differences for HbA1c. GFR was lower in proteinuric versus NA and MA patients (Table 1).

Electron Microscopy Studies
Typical diabetic glomerulopathy changes were confirmed in the type 1 diabetes groups (Table 2). Vv(Mes/glom) was greater in proteinuric, NA, and MA patients than in control subjects, and Vv(MM/glom) was increased in proteinuric compared with NA and MA patients and control subjects. Sv(PGBM/glom) was reduced in NA and proteinuric patients compared with control subjects, whereas TFS was not different among the groups. GBM width was increased in NA, MA, and proteinuric patients compared with control subject. GBM width in patients with type 1 diabetes correlated directly with AER (r = 0.65, P < 0.01) and inversely with GFR (r = −0.56, P < 0.05). Vv(Mes/glom) also correlated directly with AER (r = 0.67, P < 0.01) and inversely with GFR (r = −0.65, P < 0.05). No statistically significant associations were found between Sv(PGBM/glom) or TFS and either AER or GFR.

Light Microscopy Studies
Mean GV was numerically increased in the groups with diabetes versus control subjects, but the difference was statistically significant only between control and NA groups (Table 3). GV variability seemed to be increased in MA and proteinuric compared with NA patients and control subject. Glomerular tuft to corpuscle ratio was similar in all groups. Index of arteriolar hyalinosis was increased in patients with diabetes and was significantly higher in proteinuric patients than in other groups. Index of arteriolar hyalinosis directly correlated with AER (r = 0.62, P = 0.03), Vv(MM/glom) (r = 0.66, P = 0.02), GBM width (r = 0.76, P = 0.004), percentage of glomeruli with ATNO (r = 0.59, P = 0.04), percentage of globally sclerosed glomeruli (r = 0.87, P < 0.00001), and Vv(Int/cortex) (r = 0.65, P = 0.02) and inversely correlated with GFR (r = −0.67, P = 0.02). Vv(Int/cortex) was increased in proteinuric compared with MA patients and control subjects. Vv(PT/cortex) was decreased in proteinuric compared with NA patients. Vv(DT/cortex) was decreased in type 1 diabetes groups compared with control subjects and in proteinuric compared with NA and MA patients.

Table 2. Glomerular structural parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>GBM (nm)</th>
<th>Vv(Mes/glom)</th>
<th>Vv(MM/glom)</th>
<th>Vv(MC/glom)</th>
<th>Sv(PGBM/glom) (μm²/μm³)</th>
<th>TFS (×10⁵ μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>375 ± 65</td>
<td>0.19 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.14 ± 0.02</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>NA</td>
<td>560 ± 93b</td>
<td>0.24 ± 0.04b</td>
<td>0.12 ± 0.03</td>
<td>0.08 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>MA</td>
<td>596 ± 83c</td>
<td>0.27 ± 0.06b</td>
<td>0.15 ± 0.03</td>
<td>0.09 ± 0.03</td>
<td>0.11 ± 0.01</td>
<td>2.1 ± 1.4</td>
</tr>
<tr>
<td>P</td>
<td>761 ± 83d</td>
<td>0.39 ± 0.13c</td>
<td>0.23 ± 0.10d</td>
<td>0.09 ± 0.04</td>
<td>0.09 ± 0.03</td>
<td>1.6 ± 0.8</td>
</tr>
</tbody>
</table>

Data are mean ± SD. GBM, glomerular basement membrane; Vv(Mes/glom), fractional volume of mesangium per glomerulus; Sv(PGBM/glom), surface density of peripheral GBM per glomerulus; TFS, total filtration surface area.

bP < 0.005 versus C.

bP < 0.0005 versus C.

bP < 0.00005 versus C.

bP < 0.0001 versus C.

bP < 0.0001 versus C.

bP < 0.01 versus NA.

bP < 0.05 versus MA.

bP < 0.05 versus C.
Vv(AT/TT) was increased in proteinuric compared with NA and MA patients and control subjects. AER in patients with type 1 diabetes correlated inversely with Vv(PT/cortex) \((r = -0.72, P < 0.01)\) and directly with Vv(AT/TT) \((r = 0.72, P < 0.01)\). GFR in patients with type 1 diabetes correlated directly with Vv(PT/cortex) \((r = 0.61, P < 0.05)\) and inversely with Vv(Int/cortex) \((r = -0.83, P < 0.0001)\). GTJA were present in only 2% of glomeruli from control subjects and NA patients and 4% of glomeruli from MA patients. AG were not found in these groups (Figure 2). In contrast, GTJA were found in 71% of glomeruli in proteinuric patients. Five (8%) of the 63 glomeruli in proteinuric patients were atubular. Tip lesions were found in all glomeruli that were attached to SAT, 77% of glomeruli that were attached to LAT, and all glomeruli that were attached to ATNO (Figure 2). No tuft prolapse into proximal tubule was identified. Thickening and reduplication of Bowman’s capsules were frequent findings at the sites of adhesions, regardless of their relation to GTJ. The space between the reduplicated layers of Bowman’s capsule was filled, alone or in combination, with amorphous material, foam cells, fibroblast-like cells, and capillaries (Figure 3). In several glomeruli, this space extended toward the GTJ and beyond, seeming to dissect TBM of the

<table>
<thead>
<tr>
<th>Group</th>
<th>GV (×10⁶ μm³)</th>
<th>TCR</th>
<th>IAH</th>
<th>Vv(Int/cortex)</th>
<th>Vv(PT/cortex)</th>
<th>Vv(DT/cortex)</th>
<th>Vv(AT/TT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.2 ± 0.4</td>
<td>0.76 ± 0.04</td>
<td>0.15 ± 0.23</td>
<td>0.17 ± 0.03</td>
<td>0.62 ± 0.05</td>
<td>0.21 ± 0.03</td>
<td>0.0003 ± 0.0007</td>
</tr>
<tr>
<td>NA</td>
<td>1.9 ± 0.3⁹</td>
<td>0.75 ± 0.06</td>
<td>0.64 ± 0.66</td>
<td>0.22 ± 0.04</td>
<td>0.67 ± 0.04</td>
<td>0.12 ± 0.03⁹</td>
<td>0.0006 ± 0.0012</td>
</tr>
<tr>
<td>MA</td>
<td>1.9 ± 1.2</td>
<td>0.77 ± 0.04</td>
<td>0.67 ± 0.54</td>
<td>0.19 ± 0.05</td>
<td>0.62 ± 0.06</td>
<td>0.16 ± 0.04⁹</td>
<td>0.008 ± 0.014</td>
</tr>
<tr>
<td>P</td>
<td>2.1 ± 0.9</td>
<td>0.73 ± 0.06</td>
<td>2.0 ± 0.45⁶</td>
<td>0.28 ± 0.07⁶</td>
<td>0.54 ± 0.09⁶</td>
<td>0.08 ± 0.02⁶</td>
<td>0.043 ± 0.04⁶</td>
</tr>
</tbody>
</table>

Table 3. Stereologic parameters of renal cortex

aGV, glomerular volume \((×10⁶ μm³)\); TCR, tuft to corpuscle ratio; Vv(Int/cortex), fractional volume of interstitium per cortex; Vv(PT/cortex), fractional volume of proximal tubules per cortex; Vv(DT/cortex), fractional volume of distal tubules per cortex; Vv(AT/TT), fractional volume of atrophic tubules per total tubules.

⁹P < 0.03 versus C.
ªP = 0.0002 versus others.
被动P < 0.02 versus MA.
²P < 0.01 versus NA.
³P < 0.005 versus C.
⁴P < 0.05 versus C.
⁵P < 0.0001 versus C.
⁶P < 0.05 versus NA.
⁷P < 0.0005 versus MA.
⁸P < 0.01 versus C.
⁹P < 0.05 versus MA.
⁰P < 0.0005 versus MA.
¹P < 0.01 versus C.
²P < 0.05 versus MA.
³P < 0.01 versus C.
⁴P < 0.05 versus MA.

Figure 2. Frequency of GTJA in normoalbuminuric (NA), microalbuminuric (MA), and proteinuric (P) patients and control subjects (C). NT, normal tubules; G#, number of glomeruli.

Figure 3. A glomerulus with a tip lesion. There is an adhesion of the glomerular tuft to GTJ. The space between the reduplicated Bowman’s capsule layers (*) is filled with amorphous material. The tubular basement membrane (TBM) of the proximal portion of the proximal tubule is reduplicated (single and double arrows).
ATNO were smaller than glomeruli that were attached to NT or LAT (Table 4). Relative GV correlated inversely with IJA \((r = -0.35, P < 0.02)\) and was smaller in AG than other glomeruli \((P < 0.01)\). The tuft to corpuscle ratio was not statistically significantly different among glomeruli that were attached to NT, SAT, LAT, ATNO, and AG. Globally sclerosed glomeruli were more frequent in proteinuric \((16.9 \pm 11.7\%)\) compared with NA \((3.4 \pm 7.6\%; P < 0.05)\) and MA \((5.5 \pm 5.0\%; P < 0.05)\) patients and with control subjects \((1.8 \pm 4.0\%; P < 0.01)\).

Multiple regression analyses models with Vv(Mes/glom), TFS, and GBM width as predictors explained 67% of the GFR variability (Table 5) among the patients with type 1 diabetes. The addition of the IJA to this multiple regression model increased the GFR predictability to 75%. Glomerular structural parameters, IJA, percentage of globally sclerosed glomeruli, and Vv(Int/cortex), combined, explained 86% of the GFR variability. Vv(Mes/glom) was the only independent predictor variable in the above three models \((P < 0.005, P < 0.002, \text{and } P < 0.01, \text{respectively})\). PLRA increased the glomerular structural parameters’ explanation of GFR variability to 78% with a breakpoint at 110 ml/min. Adding IJA to the model increased the predictability of the model to 86%, whereas adding both IJA and Vv(Int/cortex) increased the predictability to 92% (Table 5). Using multiple regression analyses, Vv(Mes/glom), TFS, and GBM width explained 65% of the observed AER variability with Vv(Mes/glom) \((P < 0.005)\) and GBM width \((P < 0.05)\) as independent predictor variables. Incorporation of IJA increased the predictability of this multiple regression model to 72% with Vv(Mes/glom) \((P < 0.005)\) and IJA \((P < 0.05)\) as independent predictors. The addition of Vv(Int/cortex) did not basically change this model. It is interesting that with piecewise linear regression, glomerular structural parameters alone explained 95% of the observed AER variability (breakpoint at 52 \(\mu g/min\)), leaving almost no room for any other variable to improve the predictions (Table 5).

**Discussion**

This study presents two new findings in the pathology of DN: (1) GTJA are almost entirely restricted to proteinuric patients, and (2) the structural–functional relationships that best

**Table 4. Glomerular volume, relative glomerular volume, and tuft to corpuscle ratio of glomeruli in relation to their tubular attachments**

<table>
<thead>
<tr>
<th></th>
<th>NT (C)</th>
<th>NT (NA)</th>
<th>NT (MA)</th>
<th>NT (P)</th>
<th>SAT</th>
<th>LAT</th>
<th>ATNO</th>
<th>AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>GV (\times 10^6 \text{ (\mu m^3)})</td>
<td>1.3 ± 0.4</td>
<td>1.9 ± 0.5(^b)</td>
<td>1.9 ± 1.1(^b)</td>
<td>2.8 ± 1.1(^b,c)</td>
<td>1.9 ± 0.9</td>
<td>2.5 ± 0.8</td>
<td>1.7 ± 0.8(^d)</td>
<td>0.9 ± 0.4(^e)</td>
</tr>
<tr>
<td>RGV</td>
<td>1.01 ± 0.17</td>
<td>1.0 ± 0.19</td>
<td>1.02 ± 0.18</td>
<td>1.12 ± 0.30</td>
<td>1.03 ± 0.31</td>
<td>1.03 ± 0.41</td>
<td>0.92 ± 0.14</td>
<td>0.53 ± 0.32(^f)</td>
</tr>
<tr>
<td>TCR</td>
<td>0.76 ± 0.08</td>
<td>0.76 ± 0.06</td>
<td>0.77 ± 0.06</td>
<td>0.76 ± 0.05</td>
<td>0.77 ± 0.07</td>
<td>0.80 ± 0.05</td>
<td>0.75 ± 0.09</td>
<td>0.74 ± 0.13</td>
</tr>
</tbody>
</table>

\(^a\)RGV, relative glomerular volume; NT, normal tubules; SAT, short atrophic tubules; LAT, long atrophic tubules; ATNO, atrophic tubules with no observable opening; AG, atubular glomeruli.

\(^b\)\(^P < 0.0001\) versus NT(C).

\(^c\)\(^P < 0.0001\) versus NT(NA) and NT(MA).

\(^d\)\(^P < 0.05\) versus NT(P) and LAT.

\(^e\)\(^P < 0.05\) versus NT(P), SAT, and LAT.

\(^f\)\(^P < 0.01\) versus NT(P), SAT, LAT, and ATNO.
describe increasing AER and decreasing GFR in patients with type 1 diabetes are nonlinear.

GTJA are common in proteinuric patients with type 1 diabetes and are usually accompanied by TBCA at the GTJ (4). Adhesions at this location have been called tip lesions in steroid-resistant nephritic syndrome with focal segmental glomerulosclerosis (FSGS) and tip changes in other diseases by one group of investigators (18). We have no terminology preference but use “tip lesion” here to maintain consistency with our previous publication (4). Tip lesions have been reported in a variety of proteinuric renal diseases (5), suggesting that they are related more closely to the severity of the glomerular permselectivity defect than to the specific nature of the glomerular injury. However, proteinuria often is the clinical indication for renal biopsy, imposing a sampling bias toward more advanced disease in many glomerulopathies. This study, which included only patients who underwent research renal biopsies and which encompassed a wide range of the natural history of DN, overcame this possible selection bias. Groups in this study were matched for age and gender, and the type 1 diabetes groups also were matched for diabetes duration, thereby eliminating these potentially confounding variables. The finding that GTJA are restricted almost entirely to overtly proteinuric patients with type 1 diabetes is consistent with a watershed phenomenon in which, in association with overt proteinuria, new pathologic mechanisms of injury are induced. TBCA and FSGS, although frequent in proteinuric patients, are rare in NA and MA patients despite long and similar type 1 diabetes duration. Thus FSGS is restricted to patients who have type 1 diabetes with already established diabetic glomerulopathy. This is in contrast to experimental DN, in which in animals with glomerular hyperfiltration and capillary hypertension FSGS occurs early and

<p>| Table 5. Models of structural—functional relationships in patients with diabetes&lt;sup&gt;a&lt;/sup&gt; |
|-----------------------------------------------|-------|--------|--------|</p>
<table>
<thead>
<tr>
<th>Method</th>
<th>Predictors</th>
<th>$R^2$&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$P$</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRA</td>
<td>Vv(Mes/glom)&lt;sup&gt;b&lt;/sup&gt;, GBM width, TFS</td>
<td>0.67</td>
<td>0.001</td>
<td>GFR&lt;sup&gt;c&lt;/sup&gt; = $-262 \times Vv(Mes/glom) - 0.05 \times$ GBM width + 251</td>
</tr>
<tr>
<td>MRA</td>
<td>Vv(Mes/glom)&lt;sup&gt;b&lt;/sup&gt;, GBM width, TFS, IJA</td>
<td>0.75</td>
<td>0.001</td>
<td>GFR = $-232 \times Vv(Mes/glom) + 0.003 \times$ GBM width $- 14.5 \times IJA + 217$</td>
</tr>
<tr>
<td>MRA</td>
<td>Vv(Mes/glom)&lt;sup&gt;b&lt;/sup&gt;, GBM width, TFS, IJA, %SG, Vv(Int/cortex)</td>
<td>0.86</td>
<td>0.0005</td>
<td>GFR = $-161 \times Vv(Mes/glom) + 0.057 \times$ GBM width $- 10.9 \times IJA - 0.5 \times %SG - 163 \times Vv(Int/cortex)$</td>
</tr>
<tr>
<td>PLRA</td>
<td>Vv(Mes/glom), GBM width, TFS</td>
<td>0.78</td>
<td>0.005</td>
<td>GFR ($\geq 110$) = $-65 \times Vv(Mes/glom) - 0.12 \times GBM + 0.00005 \times TFS + 178$</td>
</tr>
<tr>
<td>PLRA</td>
<td>Vv(Mes/glom), GBM width, TFS, IJA</td>
<td>0.86</td>
<td>0.01</td>
<td>GFR ($\geq 110$) = $-71 \times Vv(Mes/glom) - 0.11 \times GBM + 0.00002 \times TFS - 15.3 \times IJA + 210$</td>
</tr>
<tr>
<td>PLRA</td>
<td>Vv(Mes/glom), GBM width, TFS, IJA, Vv(Int/cortex)</td>
<td>0.92</td>
<td>0.005</td>
<td>GFR ($\geq 110$) = $-276 \times Vv(Mes/glom) - 0.22 \times GBM + 0.0002 \times TFS - 11 \times IJA - 346 \times Vv(Int/cortex) + 200$</td>
</tr>
<tr>
<td>MRA</td>
<td>Vv(Mes/glom)&lt;sup&gt;b&lt;/sup&gt;, GBM width&lt;sup&gt;a&lt;/sup&gt;, TFS</td>
<td>0.65</td>
<td>0.0001</td>
<td>AER&lt;sup&gt;d&lt;/sup&gt;log = $7.5 \times Vv(Mes/glom) + 0.003 \times GBM + 20.3 \times TFS - 4.3$</td>
</tr>
<tr>
<td>MRA</td>
<td>Vv(Mes/glom)&lt;sup&gt;b&lt;/sup&gt;, GBM width, TFS, IJA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.72</td>
<td>0.00005</td>
<td>AER&lt;sup&gt;d&lt;/sup&gt;log = $5.4 \times Vv(Mes/glom) + 0.002 \times GBM + 11.9 \times TFS + 0.32 \times IJA - 2.7$</td>
</tr>
<tr>
<td>PLRA</td>
<td>Vv(Mes/glom), GBM width, TFS</td>
<td>0.95</td>
<td>0.01</td>
<td>AER&lt;sup&gt;d&lt;/sup&gt;log ($\geq 52$) = $8.2 \times Vv(Mes/glom) - 0.002 \times GBM + 0.00001 \times TFS - 0.85$</td>
</tr>
</tbody>
</table>

<sup>a</sup>MRA, multiple regression analysis; PLRA, piecewise linear regression analysis; IJA, index of junctional atrophy; %SG, percentage of globally sclerosed glomeruli; AER<sup>d</sup>log, logarithmic albumin excretion rate; $R^2$a, adjusted $R^2$.

<sup>b</sup>Independent predictor variable.

<sup>c</sup>ml/min.

<sup>d</sup>μg/min.
in the absence of prominent glomerular extracellular matrix accumulation (19,20). Thus, FSGS lesions in experimental models of diabetes are distinctly different in their natural history from those described here, and this suggests the need for extreme caution in extrapolating from these animal models to human DN. These observations also are consistent with the natural history of DN in type 1 diabetes. Patients who are destined to develop overt proteinuria begin with a long silent period during which AER is normal or near normal for 10 to 30 yr or longer and GFR is normal or high. Renal lesions that develop during this time may, in fact, overlap in severity with those regularly seen in MA and in proteinuric patients, as in the present and previous studies (3). Once persistent MA develops, lesions are, on average, worse than in NA patients, and this functional change indicates a substantially increased risk for progression to proteinuria (2,3,21,22). However, GFR generally is preserved until proteinuria typically usters in a progressive GFR decline toward ESRD. We hypothesize that a combination of podocyte and proximal tubular epithelial cell injuries in diabetes (23–25) makes the GTJ area vulnerable to synchia. Once TBCA happens, paraglomerular filtration into and beyond Bowman’s capsule could lead to Bowman’s capsule thickening, reduplication/dissection, and periglomerular fibrosis (26–28). This aberrant filtrate flow, perhaps extending toward the GTJ and dissecting the proximal portion of the proximal TBM, could lead to GTJA, proximal tubular atrophy, and AG.

This study also demonstrated that simple linear models do not best describe structural–functional relationships in patients with type 1 diabetes over a wide range of AER and GFR. PLRA provided models with substantially higher predictive values. Given the relatively small number of patients in this study, predictability could have been overestimated. Therefore, the robustness of the provided models can be determined only by additional studies. Nevertheless, these analyses are in accordance with the natural history of the human DN and with the hypothesis that both GTJA and downstream tubulointerstitial injury are accelerated by the development of high-grade glomerular permselectivity defect. Vv(Int/cortex) was increased only in the proteinuric patients, accompanied by a decrease from control subjects in Vv(PT/cortex) and Vv(DT/cortex) and accompanied by an increase in the volume fraction of atrophic cortical tubules, consistent with previous findings (4) and with the hypothesis that proteinuria has noxious downstream consequences to the nephron (29,30).

All type 1 diabetes groups had glomerular enlargement, a feature not seen with shorter diabetes durations (14,31). The glomerular urinary space, however, was not disproportionately increased, despite GTJA, which conceivably could obstruct the flow of filtrate from Bowman’s space to the proximal tubule. This may not reflect the in vivo reality or could suggest that filtrate could leave through paraglomerular filtration into and across Bowman’s capsule, as suggested above. Glomeruli that are attached to normal tubules in proteinuric patients are larger than those that are attached to normal tubules of patients in the other type 1 diabetes groups, perhaps representing compensatory hypertrophy. However, as described elsewhere, AG in proteinuric patients are reduced in volume, whereas glomeruli with ATNO are of intermediate size (4).

Conclusion

The findings of this study demonstrate that TBCA and GTJA are essentially restricted to patients with type 1 diabetes with overt proteinuria and, along with interstitial expansion, augment diabetic glomerulopathy’s explanation of GFR loss in DN. Models that allow for increased rates of GFR decline in the later stages of the evolution of DN lesions provide a better fit to the data than simpler linear models. New mechanisms of nephron injury seem to be induced by overt proteinuria. It is tempting to hypothesize that therapies that reduce proteinuria also may reduce GFR decline in DN by limiting the damaging influence of proteinuria on the GTJ and tubulointerstitium, but this remains to be determined.

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References


