Structural-Functional Relationships in Alport Syndrome

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ABSTRACT
Renal morphometric analysis was performed in 15 (13 male) Alport syndrome patients ages 4 to 26 years, along with 10 controls ages 3 to 26 years, to better understand the structural basis of renal dysfunction in Alport syndrome. The glomerular basement membrane (GBM) width class frequencies of controls were normally distributed; those of Alport syndrome patients were slightly skewed, especially toward thicker classes, although there was also an increase in the proportion of thinner classes. Mesangial volume fraction was not different between Alport syndrome patients (0.21 ± 0.09) and controls (0.19 ± 0.04). There was an inverse correlation between mesangial volume fraction and creatinine clearance in Alport syndrome patients (r = -0.72, P < 0.01); however, the creatinine clearances in Alport syndrome patients were far less than in insulin-dependent diabetic patients. The cortical interstitial volume fraction was highly inversely correlated with creatinine clearance in Alport syndrome patients (r = -0.85, P < 0.01). Global glomerular sclerosis was 0% in five and 5 to 61% in nine Alport syndrome patients and correlated inversely with creatinine clearance (r = -0.74, P < 0.01). However, the creatinine clearance was lower in Alport syndrome than in diabetic patients with similar cortical interstitial volume fraction and percent glomerular sclerosis. There was no significant difference in an index of glomerular number between Alport syndrome patients and controls. Thus, changes in mesangial volume fraction, cortical interstitial volume fraction, percent glomerular sclerosis, and surface density of the peripheral GBM in Alport syndrome patients only partially account for the reduction in creatinine clearance. It was speculated that decreased glomerular capillary wall hydraulic conductivity in Alport syndrome could explain many of these observations.

Key Words: Morphometry, end-stage renal failure, glomerular basement membrane, mesangium, interstitium

Guthrie, in 1902 (1), described recurrent hematuria in several members of a British family in which Alport subsequently reported the occurrence of renal failure and deafness (2). Since that time, Alport syndrome has emerged as an important hereditary form of progressive nephritis, characterized by recurrent hematuria and progressive renal failure in which neurosensory hearing loss and ocular abnormalities occur with varying frequency (3). Recently, scientific interest in this syndrome has been rekindled by new insights into the molecular basis of this disorder. Mutations of the X-chromosomal gene COL4A5, which encodes the a5 chain of Type IV collagen, are found in many Alport kindreds and appear to result in defective expression of the a5 chain of Type IV collagen in the kidney (4). Despite these new insights, there is an incomplete understanding of the processes leading to the development of progressive renal lesions in Alport syndrome patients (5), nor is it clear which lesions are most closely related to the ultimate development of renal functional deterioration in this disease.

Initially, Alport syndrome was thought to be a primary chronic tubulointerstitial nephritis and the finding of interstitial foam cells was considered to be suggestive of the diagnosis (6). Since the advent of the transmission electron microscope, various glomerular lesions have been described, and these allow more...
accurate recognition of the syndrome (6–8). However, careful quantitation of both glomerular and interstitial structure in relation to renal function has not been done in Alport syndrome. Thus, we performed light and electron microscopic morphometric analysis of renal structure in Alport syndrome patients, and the findings were compared with values in normal kidneys and were related to the functional consequences of the renal lesions of diabetes mellitus.

MATERIALS AND METHODS

Patients

We performed a retrospective study of 15 patients (13 males) with Alport syndrome ranging from 4 to 26 yr of age (median, 12 yr; mean, 13.3 yr) (Table 1). Eleven patients were from University of Minnesota Hospital, one patient was from Hennepin County Medical Center (Minneapolis, MN), and three patients were from Hôpital Necker-Enfants Malades (Paris, France). Thirteen patients had sensorineural hearing loss. All had a family history of kidney disease, and nine had biopsy-proven Alport syndrome in their family (Table 1). All studies in Alport syndrome patients were performed for clinical reasons and after informed consent had been obtained. Before renal biopsy, all patients had one or two 24-h urine collections for creatinine clearance (expressed as milliliters per minute per 1.73 m²) and urinary protein excretion (expressed as grams per day per square meter) by standard laboratory procedures (Table 1). Glomerular permeability to protein was estimated by dividing the protein excretion in micromilligrams per minute by raw creatinine clearance in milliliters per minute. Blood pressure data were based on repeated determinations obtained during hospitalization for renal biopsy. Definitions of hypertension derived from the Evaluation and Treatment of High Blood Pressure Control (9) and the Task Force on Blood Pressure Control in Children (10) were used. A percutaneous renal biopsy was performed for clinical indication after informed consent was obtained from each patient or the responsible parent.

In order to compare the relative severity of the glomerular and interstitial lesions of Alport syndrome with that of other renal disorders, we used the data from insulin-dependent diabetes mellitus patients studied at our institution. Thus, we compared functional relationships in Alport syndrome and diabetic patients with structural measures of volume density of the cortical interstitium (11), percent global glomerular sclerosis (12), mesangial fractional volume, and surface density of the PGBM (unpublished data). For the comparison of the structural measure of the volume density of the cortical interstitium, we used previously published insulin-dependent diabetic patient material (11). This comprised 84 patients (69 women) age 31 ± 7 (30; 17 to 56) [mean ± SD (median, range)] yr with diabetes of 22 ± 7 yr duration. Creatinine clearance was 102 ± 27 (99, 55–176) mL/min per 1.73 m². Fortye-five patients had normal albumin excretion rates (<22 mg/24 h); 17 patients had microalbuminuria (22 to 220 mg/24 h), and 34 had overt proteinuria (>220 mg/24 h). Four of 45 normoalbuminuric, 7 of 17 microalbuminuric, and 18 of 34 overtly proteinuric patients had hypertension. For the comparison of the structural measure of percent glomerular sclerosis, we used previously published diabetic patient material (12). This comprised 43 patients (29 women) age 31 ± 7 (31, 17 to 55) yr with duration of diabetes of 20 ± 6 (21, 7 to 32) yr. Creatinine clearance was 91 ± 25 (86, 47 to 139) mL/min per 1.73 m². Sixteen were normoalbuminuric, 6 were microalbuminuric, and 21 were overtly proteinuric. Eighteen were hypertensive.

For the comparison of the structural measures of mesangial fractional volume and PGBM surface density, we used a group of 62 diabetic patients (41 women) age 33 ± 9 (32, 17 to 60), with a duration of diabetes of 19 ± 8 yr (18, 9 to 57) and a creatinine clearance of 101 ± 27 (102, 38 to 173) mL/min per 1.73 m². Thirty-six had normal albumin excre-

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<th>Estimated Permeability to Protein (μg/mL Ccr)</th>
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Abbreviations: Ccr, creatinine clearance; —, not available.

Note: cases are listed in order of ascending values for Ccr. a ESRD at the time of biopsy.
tion, 12 had microalbuminuria, and 14 had overt proteinuria. Sixteen were hypertensive. There was overlap between these three cohorts of diabetic patients. Additionally, we compared relationships of estimated glomerular permeability with protein and filtration slit length density in Type I mesangiocapillary glomerulonephritis (unpublished data) and Alport syndrome. There are nine (four female) Type I mesangiocapillary glomerulonephritis patients ages 11 ± 5 (9, 6 to 20) yr. Their creatinine clearance at biopsy was 77 ± 46 (82, 10 to 129) mL/min per 1.73 m², and three patients were hypertensive (13). Urinary total protein excretion was compared relationships of estimated glomerular permeability with protein and filtration slit length density in Type I mesangiocapillary glomerulonephritis (unpublished data) and Alport syndrome. There are nine (four female) Type I mesangiocapillary glomerulonephritis patients ages 11 ± 5 (9, 6 to 20) yr. Their creatinine clearance at biopsy was 77 ± 46 (82, 10 to 129) mL/min per 1.73 m², and three patients were hypertensive (13). Urinary total protein excretion was 0.82 (0.07 to 163) g/m² per 24 h.

Control renal biopsies were obtained at the time of donation for kidney transplantation from 10 (seven male, three female) normal individuals age matched to the Alport syndrome patients. The normal controls were ages 4 to 26 yr (median, 12.5 yr; mean, 13.2 yr). Three biopsies came from living related donors (all above 18 yr of age) and seven came from cadaver donors, six below 18 yr of age. The baseline renal allograft tissues were obtained in the operating room at the time of transplant surgery by needle biopsy after informed consent was obtained from the transplant recipients or their parents. We have previously demonstrated that morphometric values derived from living related donors and cadaver donors are not different (14).

To estimate glomerular number, we selected four Alport syndrome patients who had normal to slightly decreased creatinine clearance at the time of biopsy who also had intravenous pyelograms (Patients 12 to 15). An additional four Alport syndrome patients from Hôpital Necker-Enfants Malades (Paris, France) who had normal creatinine clearance or serum creatinine at the time of intravenous pyelography and renal biopsy were also included in this component of the study. Nine living related kidney donors, who had intravenous pyelograms during donor evaluation and who had renal biopsies performed at the time of donation, were selected as normal controls.

**Tissue Processing**

Part of the renal biopsy specimen was placed in Zenker’s fixative, washed, embedded in paraffin, and serially sectioned at approximately 4 μm. Sections were stained with periodic acid–Schiff and examined for percent glomerular sclerosis, interstitial volume fraction, mean glomerular volume, and Vv (glomerulus/cortex) as described below. The other part of the tissue was cut into approximately 1-mm cubes, placed in 2.5% glutaraldehyde in 0.17 M cacodylate buffer, and prepared for electron microscopy as previously described (15).

Thick (1-μm) sections were cut from each electron microscopy block, stained with toluidine blue, and used for the selection of the centernost glomerulus in each block in which the entire profile was at least one tubular diameter from the edge of the block. Three such glomeruli were selected from each biopsy for thin sectioning, except in Patient 8, where only two glomeruli fit the above criteria. Thin sections were stained with uranyl acetate and lead citrate for examination with a JEOL 100 CX electron microscope (JEOL, Tokyo, Japan). Each glomerular cross-section was photographed in its entirety at a magnification of approximately ×3,500. Micrographs were placed together to form a montage of the entire glomerular cross-section. Also, each glomerulus was entered randomly, and 20 to 40 evenly spaced electron micrographs were systematically taken throughout the glomerulus at an approximate final magnification of ×22,000. A calibration grid (2,160 lines/mm) was photographed with each glomerulus to determine the final magnification.

**Morphometric Analysis**

**Light Microscopy.** Mean glomerular volume was measured on the light microscopic sections at an approximate magnification of ×150 using the point-counting method of Weibel and Gomez (16). Five to 65 (median, 16) glomerular profiles per patient were measured.

**Percent Sclerosed Glomeruli.** Glomeruli were considered to have global sclerosis if all of the glomerular tufts were totally replaced by dense scar or exhibited wrinkling of the GBM and marked irregularity and collapse of the capillary luminal spaces. Globally sclerosed glomeruli were usually associated with other abnormalities, including fibrous crescents, periglomerular fibrosis, tubular atrophy, and splitting or reduplication of tubular basement membranes. The number of sclerosed glomeruli was then expressed as a percentage of the total (12). Five to 83 (median, 18) glomeruli per Alport syndrome patient and 20 to 99 (median, 40) per diabetic patient were examined. Glomerular volume and percent sclerotic glomeruli were not performed in one patient (Table 2; Patient 8) because of inadequate numbers of glomeruli.

**Volume Density of the Cortical Interstitium.** This was determined on the light microscopic sections at an approximate magnification of ×300 by point-counting images projected onto a white surface with a projection microscope (11). Fine points were counted to determine the number of points falling on the interstitium (P₀), defined as points falling other than on glomeruli, tubules, and vessels larger than tubules. Coarse points were counted to determine points falling on the renal cortex (P₁). Because each coarse point defined four fine points, then

\[
V_v (\text{interstitium/cortex}) = \frac{P₀}{P₁} \times 4 \text{ (m}^3/\text{mm}^3)\]

The cortical interstitial volume fraction was examined independently by two observers (K.H.K. and P.H.L.). The results from the two observers were closely matched (y = 1.0x + 1.2; r = 0.95), and the average of the data pair from each biopsy was used as the result.

**Index of Glomerular Number.** We obtained an estimate of glomerular number (index) by multiplying the cortical volume of a kidney by the fraction of renal cortex made up of glomeruli (Vv [glomerulus/cortex]) and dividing this by the mean glomerular volume for that kidney ×10⁶. Cortical volume per kidney was obtained as follows. We determined kidney volume from intravenous pyelography using the method of Moell (17). According to Wiltraks (18), the fraction of kidney volume that is cortex is the same in diseased and in normal kidney and averages 0.56. We thus estimated cortical volume to be 0.56 × kidney volume. The volume density of glomeruli per cortex (Vv [glomerulus/cortex]) was determined on light microscopic sections at an approximate magnification of ×150 by point counting images projected onto a white surface with a projection microscope. The number of fine points hitting glomeruli and the number of coarse points hitting cortex in the entire cortical region of a section were counted. Vv [glomerulus/cortex] was calculated as follows:

\[
V_v (\text{glomerulus/cortex}) = \frac{P_{glomeruli}}{P_{cortex} \times 16}
\]
TABLE 2. Morphometric data in Alport syndrome and normal controls

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<th>Global Glomerular Sclerosis (%)</th>
<th>Volume Density (Interstitium/Cortex) (%)</th>
<th>Mesangial Volume/ Glom (×10⁶ μm³)</th>
<th>Volume Density Mesangium/ Glom (μm²/μm³)</th>
<th>Surface Density (PGBM/Glon) (μm²/μm²)</th>
<th>PGBM Surface Area/Glon (10³ μm²)</th>
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Normal Values

| Mean       | 0.86                                   | 7                              | 0.17                                   | 0.19                           | 0.13                                     | 112.7                          | 1.75                           | 205.0                           |
| SD         | 0.3                                    | 1                              | 0.07                                   | 0.04                           | 0.13                                     | 6.7                            | 0.31                           | 95.0                             |
| P          | <0.025                                 | <0.005                         | <0.05                                  | NS                             | NS                                       | <0.05                          | <0.001                         | NS                              |

Abbreviations: Glom, glomerulus; NS, not available; NS, not significant.

Pglomerulus is the number of fine points overlying glomeruli, and P_cortex is the number of coarse points overlying cortex. There were 16 fine points for each coarse point on the grid. Glomerular volume was estimated by the method of Weibel and Gomez (16) and as reported from our laboratory (19). The number of glomeruli per kidney was estimated by the following equation.

Index of glomerular number =

\[
\frac{\text{renal cortical volume} \times V_v (\text{glomerulus/cortex})}{\text{mean glomerular volume} \times 10^6}
\]

Electron Microscopy

Routine stereologic techniques, previously described in detail (14,19,20), were used to measure mesangial fractional volume, \(V_v\) (mesangium/glomerulus), \(V_v\) (mesangial cell/mesangium), \(V_v\) (mesangial matrix/mesangium), and surface density of the PGBM. Values for a given glomerulus were weighted for the glomerular tuft area examined. Mesangial volume fraction and surface density of the PGBM were measured on the montages, whereas GBM measurements, \(V_v\) (mesangial cell/mesangium), and \(V_v\) (mesangial matrix/mesangium) and were obtained with the high-power \((\times 22,000)\) photomicrographs. The coefficient of variation among three (or more) glomeruli for the measure of mesangial volume fraction is 14%, and for surface density of the peripheral GBM, it is 17% (20,21). Because of the irregularity of the GBM in Alport syndrome patients, GBM width was not estimated. Usual estimates involve measuring classes of widths orthogonal to a set of lines intercepting the GBM with a superimposed grid (22). The measurements are done with a harmonic ruler, which defines the classes and provides lower weight to wider GBM segments. This considers that tangential cuts can only make the GBM wider than the true width, which would be provided by perpendicular sections through the GBM. Instead of measuring the harmonic mean GBM width, we elected to count the frequency (%) of width classes which would be provided by perpendicular sections through the GBM. Instead of measuring the harmonic mean GBM width, we elected to count the frequency (%) of width classes which would be provided by perpendicular sections through the GBM. Instead of measuring the harmonic mean GBM width, we elected to count the frequency (%) of width classes which would be provided by perpendicular sections through the GBM.
length per glomerulus was calculated from slit length density per area and peripheral capillary filtration surface area.

Statistics

A two-tailed t test was used to compare the differences between the means of Alport syndrome patients and controls. Values for glomerular volume or percent glomerular sclerosis were weighted in the analyses for the number of glomeruli studied. Relationships between renal structural and functional values were examined by linear regression by the method of least squares. Comparisons of regression lines between the Alport syndrome group and other disease groups were performed by regression analysis with dummy variables. Because the values for estimated glomerular permeability to protein were not normally distributed, these data were log transformed for statistical analysis. Values of P < 0.05 were considered to be statistically significant.

RESULTS

Patient Characteristics

Creatinine clearance ranged from 5 to 162 mL/min per 1.73 m², and urinary protein excretion ranged from 0 to 4.2 g/day per m² (Table 1). The seven patients who were hypertensive had creatinine clearances of less than 70 mL/min per 1.73 m². The renal tissues studied from three patients (patients 1 to 3) were from nephrectomy specimens. Follow-up data on 2 patients were not available, whereas 10 patients were monitored for 1 to 12 yr. Six of these 10 patients developed ESRD 8 to 140 months after the biopsy (Table 1).

Renal Structure in Alport Patients Versus Controls

The mean glomerular volume was greater in Alport syndrome patients than in controls (P < 0.025) (Table 2). The cortical interstitial volume fraction was increased in all patients, markedly in many (P < 0.005) (Table 2).

The frequency distribution of GBM width classes showed a shift toward wider measurements in the Alport syndrome patients than in the controls, with a lesser tendency toward an excess of thinner classes in these patients (Figure 1). This is reflected in the increased skewness of the distribution of GBM width classes in the Alport syndrome patients than in controls (P < 0.01) (Figure 1). The variance of the individual measures was greater in the Alport patients than in controls (P < 0.005), indicating greater irregularity in GBM structure.

The mesangial volume fraction was not different between Alport syndrome patients and controls. However, because of glomerular enlargement, mesangial volume/glomerulus was increased in Alport syndrome patients compared with controls (P < 0.05; Table 2). There was no significant difference in the surface density of the PGBM in Alport syndrome patients compared with controls, but PGBM surface area/glomerulus was increased in Alport syndrome patients (P < 0.05), again as the result of increased glomerular volume (Table 2).

The filtration slit length density per PGBM surface (micrometers per square micrometers) was significantly decreased in Alport syndrome patients versus controls (P < 0.001; Table 2). However, because of glomerular enlargement, filtration slit length (×10³ μm) per glomerulus was not significantly different in Alport syndrome patients compared with the controls (Table 2).

The values obtained for estimating glomerular number in Alport patients and controls are presented in Table 3. Because of their older age, the kidney volumes and mean glomerular volumes of the controls were greater than those of the Alport syndrome patients. The mean estimated glomerular number per patient was 3.1 ± 1.4 in the eight Alport syndrome patients and 4.4 ± 1.2 in the nine controls (not significant; Table 3).

Structural-Functional Relationships in Alport Syndrome Patients

Mesangial volume fraction correlated inversely with creatinine clearance (r = −0.72, P = 0.003; Figure 2). Surface density of the PGBM correlated directly with creatinine clearance (r = 0.71, P = 0.003; Figure 3), but PGBM surface area per glomerulus did not. However, total estimated PGBM surface area per patient correlated directly with creatinine clearance (r = 0.57, P = 0.03).

Creatinine clearance and cortical interstitial volume fraction were inversely correlated (r = 0.85; P = 0.0001; Figure 4). Global glomerular sclerosis was 0% in five and 5 to 61% in nine Alport syndrome patients and correlated inversely with creatinine clearance (r = −0.74, P = 0.002; Figure 5). There was no significant relationship between glomerular volume and creatinine clearance.

Total slit pore length per patient correlated directly with creatinine clearance (r = 0.72, P = 0.0041). Filtration slit length density per PGBM was inversely related to the log of estimated protein permeability.
Figure 2. Relationships between mesangial volume fraction and creatinine clearance in Alport syndrome \((r = -0.72; P < 0.01)\) and insulin-dependent diabetes mellitus (IDDM) \((r = -0.70; P < 0.01)\). The slopes of the regression lines for Alport syndrome \((-470.01)\) and diabetic patients \((-182.45)\) are significantly different \((P < 0.01)\).

(micrograms of protein per milliliter of creatinine clearance) \((r = 0.67, P = 0.02; \text{Figure } 6)\).

Structural-Functional Relationships in Alport Syndrome Compared With Diabetic Nephropathy and Mesangiocapillary Glomerulonephritis Type 1

The relationships of glomerular and interstitial lesions to functional change in Alport syndrome were compared with those relationships in two other renal disorders—diabetic nephropathy and Type 1 mesangiocapillary glomerulonephritis. Mesangial volume fraction correlated inversely with creatinine clearance in both diabetic \((r = 0.72, P = 0.0001)\) and Alport syndrome patients (see above). However, for a given increase in Vv (mesangium/glomerulus), creatinine clearance was greater in the diabetic compared with

![Figure 6](image-url)
the Alport syndrome patients. Further, as shown in Figure 2, the slopes of the regression for Alport syndrome (−470) and diabetic patients (−182) were significantly different ($P < 0.01$). Similarly, there was a greater reduction in creatinine clearance as related to the declining surface density of the PGBM in Alport syndrome compared with diabetic patients. The slopes and intercepts of the regression lines for Alport syndrome (882.4, −41.85) and diabetic (431.37, 53.70) patients were significantly different ($P < 0.01$, $P < 0.01$) (Figure 3). The patients with diabetes also had greater values of creatinine clearance for any given measures of cortical interstitial volume fraction than the Alport syndrome patients. Although the slopes of the regression lines for these two correlations were similar, the intercept was greater in the diabetic patients (Alport syndrome, 129.4; diabetes, 153.5; $P < 0.01$; Figure 4). The slopes of the regression lines for the relationship of percent global sclerosis and creatinine clearance were also different between Alport syndrome (−1.19) and diabetic patients (−0.95, $P < 0.01$). Again, creatinine clearance for a given value of percent global sclerosis tended to be greater in the diabetic patients (Figure 5). In contrast, the decrease in filtration slit length density per PGBM area as related to estimated protein permeability was similar in Alport syndrome patients ($r = 0.74$, $P < 0.01$) and mesangiocapillary glomerulonephritis Type I patients ($r = −0.94$, $P < 0.01$; Figure 6).

**DISCUSSION**

Although the characteristics of the renal lesions of Alport syndrome are well known, careful quantitative analyses and structural-functional relationships have not previously been performed. Although tubulointerstitial injury in Alport syndrome was well described many years ago and although glomerular injury has received more attention recently, the question as to which of these abnormalities is more closely related to the progressive loss of kidney function in Alport syndrome has heretofore not been addressed.

Previous studies have reported thin GBM widths in younger Alport syndrome patients and thickening of the GBM in older patients. However, because of the marked irregularity of the GBM in Alport syndrome, we did not consider it valid to estimate mean GBM width. The increase in the variance of the individual width measurements that was documented in Alport syndrome is probably an indirect measure of this irregularity, whereas the increased skewness is associated with increased numbers of GBM intercepts in

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**Figure 4.** Relationship between cortical interstitial volume fraction and creatinine clearance in Alport syndrome ($r = −0.85$, $P < 0.01$) and insulin-dependent diabetes mellitus (IDDM) patients ($r = −0.64$, $P < 0.01$). The slopes of the regression lines for Alport syndrome (129.38) and diabetes (153.45) are significantly different ($P < 0.01$). Diabetic data from PH Lane et al. (11), with permission.

**Figure 5.** Relationship between global glomerular sclerosis and creatinine clearance in Alport syndrome ($r = −0.74$, $P < 0.01$) and insulin-dependent diabetes mellitus (IDDM) patients ($r = −0.64$, $P < 0.01$). The slopes of the regression lines for Alport syndrome (−1.79) and diabetes (−0.95) are significantly different ($P < 0.01$). Diabetic data from RD Harris et al. (12), with permission.

**Figure 6.** Relationships between filtration slit length density per PGBM area and estimated protein permeability in Alport syndrome ($r = −0.74$, $P < 0.01$) and Type I mesangiocapillary glomerulonephritis (MCGN) patients ($r = −0.94$, $P < 0.01$). Note log scale for estimated protein permeability. Ccr, creatinine clearance.
the highest and, to a lesser extent, in the lowest classes of measurements in the Alport syndrome patients, regardless of age. Thus, our study documents a greater increase in thickened as compared with thinner areas of GBM in Alport syndrome patients. Others had previously described additional features of the fine structural changes in the GBM in Alport syndrome including basket weaving and lamellation of the GBM (3,6–8). Gaboardi et al. (25) found that the percentage of the PGBM showing changes of basket weaving was directly correlated with the magnitude of proteinuria. These studies suggest that proteinuria in Alport syndrome may be secondary to GBM alterations.

The mesangium early in the course of Alport syndrome has been described as normal, whereas with time, there has been said to be increased mesangial matrix and cells (25,26). Mesangial volume fraction was not different between Alport syndrome patients and controls in our study, but because of the increased glomerular volume in Alport syndrome patients, mesangial volume per glomerulus was increased. Although there was an inverse relationship between mesangial volume fraction and creatinine clearance in the Alport syndrome group, the values for mesangial volume fraction in Alport syndrome patients were far less than those of diabetic patients or patients with Type I mesangiocapillary glomerulonephritis (15) with similar creatinine clearance. Mesangial volume per glomerulus did not correlate with creatinine clearance in Alport syndrome, and these values were also smaller than those of diabetic patients (unpublished data) or Type 1 mesangiocapillary glomerulonephritis (13).

There was no significant difference in the surface density of the PGBM in Alport syndrome patients and controls. Although correlated with creatinine clearance in Alport syndrome, the values for the surface density of the PGBM in Alport syndrome were much higher than those of diabetic or Type I mesangiocapillary glomerulonephritis patients with similar creatinine clearance (data not shown). Although the surface density of the PGBM correlated with creatinine clearance, the PGBM area per glomerulus did not. The total peripheral capillary filtering surface per patient correlated with creatinine clearance. Again, the values for this estimate of available glomerular filtration surface were greater in Alport syndrome than in diabetic patients, especially in those with moderately to severely impaired renal function.

In the later stages of Alport syndrome, segmental glomerular sclerosis appears to lead to global glomerular sclerosis. Global glomerular sclerosis correlated inversely with creatinine clearance in Alport syndrome patients, but percent glomerular sclerosis was less in Alport syndrome patients than in diabetic patients with similar creatinine clearance (12). Thus, the severity of the glomerular lesions associated with a decline in GFR in Alport syndrome patients was much less than that seen in other glomerular disorders such as diabetic nephropathy or mesangiocapillary glomerulonephritis Type I. Perhaps this was to be expected in a disorder reputed to have important tubulointerstitial lesions. Surprisingly, however, although the cortical interstitial volume fraction was highly inversely correlated with creatinine clearance in Alport syndrome patients, the values of cortical interstitial volume fraction were less in Alport syndrome compared with diabetic patients with similar creatinine clearance levels (11).

Thus, although mesangial volume fraction, cortical interstitial volume fraction, percent glomerular sclerosis, and surface density of the PGBM were all correlated with creatinine clearance in Alport syndrome, the abnormalities in these measures were less than in diabetic patients with similar creatinine clearance, indicating that these changes only partially explain the reduced creatinine clearance in Alport syndrome. Therefore, this study has uncovered an important question regarding the nature of the abnormalities responsible for the reduction in GFR in Alport syndrome. We considered the possibility that Alport syndrome patients have an inherent reduction in nephron number, but our preliminary efforts to estimate this parameter do not support this hypothesis. Our estimates of glomerular number were higher in Alport syndrome patients and controls than those reported recently by Nyengaard and Bendtsen (27). Both studies showed similar measures of the total volume of glomeruli per patient in normal patients, but the glomerular volume in our study was lower than that of Nyengaard and Bendtsen because of systematic technical factors discussed in detail in their article (27). For this reason, we report these data as an index of glomerular number, providing a basis for relative comparisons between Alport syndrome patients and controls. We believe this comparison to be reasonable because the systematic factors resulting in different estimates of glomerular number in the two studies should in no way invalidate the comparison of the indices of glomerular number of Alport syndrome patients and controls because these factors would be identical in both groups. Another possibility is that there are increased numbers of atubular glomeruli in Alport syndrome patients (28), but this is not likely because atubular glomeruli tend to be small (28) and mean glomerular volume is increased in Alport syndrome patients. Nonetheless, this possibility cannot be excluded without performing careful studies of serially sectioned glomeruli—studies requiring prospective planning of tissue-sectioning strategies. Another hypothesis could account for our observations. Although highly speculative, it is possible that the GBM biochemical abnormalities in Alport syndrome lead to a decrease in the hydraulic conductivity of the glomerular capillary. If this speculation is correct, it might explain certain aspects of the progressive nephron injury associated with Alport syndrome. Reduced glomerular capillary wall hydraulic conductivity could trigger adaptive responses, such as in-
increased glomerular capillary flows and pressures that could be injurious to the glomerulus (29). The glomerular susceptibility to hemodynamic injury in this disorder could be particularly important if the biochemical defect of Alport syndrome adversely influences the structural resilience of the glomerular basement membrane. Conceivably, this could result in basement membrane basket weaving, thinning, and tears, which could ultimately lead to proteinuria and focal and global glomerular sclerosis. Although these hypotheses may be difficult to test, they could suggest therapeutic alternatives that might prevent or delay the progression of this disease.

Changes in epithelial cell structure have frequently been described in terms of foot process widening. However, epithelial foot processes consist of primary, secondary, and tertiary structures, the width of which is not normally distributed (30). Filtration slit length density, an indirect estimate of epithelial cell structural integrity, avoids this problem, and we have previously shown this measure to be closely inversely correlated with albumin excretion rate in diabetic patients (30). Because albuminuria was not measured in our Alport syndrome patients and because the Alport syndrome patients had a very wide range of GFR as estimated by creatinine clearance, we used protein excretion per milliliter of creatinine clearance as a measure of permeability. It has been reported that epithelial foot process changes may be extensive, even in the absence of significant proteinuria in Alport syndrome patients (31). However, in this study, estimated protein permeability was closely and inversely related to filtration slit length density. Further, this relationship was almost identical to that of patients with mesangiocapillary glomerulonephritis Type I (M. Hattori, unpublished data). Similar relationships of slit pore length density and permeability to protein have been noted in membranous nephropathy (32) and minimal lesion nephrotic syndrome (33). The common relationship of proteinuria and epithelial cell structural disturbance in disorders of such disparate pathogenesis as Alport syndrome, diabetic nephropathy, mesangiocapillary glomerulonephritis Type I, minimal lesion nephrotic syndrome, and membranous nephropathy is consonant with the hypothesis that these epithelial cell changes are, in large part, secondary to the proteinuria. However, other explanations such as common causes of proteinuria and epithelial cell structural changes are also possible, and the work presented here cannot resolve these questions. In summary, the main finding of this report is that the severity of the glomerular and interstitial abnormalities do not, alone, adequately explain the progressive reduction in GFR in Alport syndrome.

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