Histopathology of Diabetic Nephropathy

Paola Fioretto, MD, PhD, and Michael Mauer, MD†*

Summary: The clinical manifestations of diabetic nephropathy, proteinuria, increased blood pressure, and decreased glomerular filtration rate, are similar in type 1 and type 2 diabetes; however, the renal lesions underlying renal dysfunction in the 2 conditions may differ. Indeed, although tubular, interstitial, and arteriolar lesions are ultimately present in type 1 diabetes, as the disease progresses, the most important structural changes involve the glomerulus. In contrast, a substantial subset of type 2 diabetic patients, despite the presence of microalbuminuria or proteinuria, have normal glomerular structure with or without tubulointerstitial and/or arteriolar abnormalities. The clinical manifestations of diabetic nephropathy are strongly related with the structural changes, especially with the degree of mesangial expansion in both type 1 and type 2 diabetes. However, several other important structural changes are involved. Previous studies, using light and electron microscopic morphometric analysis, have described the renal structural changes and the structural-functional relationships of diabetic nephropathy. This review focuses on these topics, emphasizing the contribution of research kidney biopsy studies to the understanding of the pathogenesis of diabetic nephropathy and the identification of patients with a higher risk of progression to end-stage renal disease. Finally, evidence is presented that the reversal of established lesions of diabetic nephropathy is possible.

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The constellation of the renal structural lesions occurring in diabetes is unique, although each of these lesions can be observed individually in other renal disorders. The morphologic lesions in type 1 diabetes mellitus (T1DM) predominantly affect the glomeruli, with thickening of the glomerular basement membrane (GBM) and mesangial expansion, although the podocytes, renal tubules, interstitium, and arterioles also undergo substantial changes, especially at later stages of disease.[1-5]

*Department of Medical and Surgical Sciences, University of Padova Medical School, Padova, Italy

†Pediatric Nephrology, University of Minnesota Medical School, Minneapolis, MN

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Address reprint requests to Paola Fioretto, Department of Medical and Surgical Sciences, University of Padova, Via Giustiniani 2, 35128 Padova, Italy. E-mail: paola.fioretto@unipd.it

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GBM thickening, the first measurable change, has been detected as early as 1.5 to 2.5 years after the onset of T1DM.[6,7] Thickening of the tubular basement membrane (TBM) closely parallels that of GBM thickening, implying that glomerular hemodynamic perturbations are not required for these changes to occur.[3] Mesangial expansion, predominantly caused by an increase in mesangial matrix, develops later, although an increase in the matrix component of the mesangium can be detected as early as 5 to 7 years after the onset of diabetes.[8-10] Thereafter, these structural changes do not necessarily develop at the same rate in individual patients.[11] In fact, although GBM thickening may develop steadily over time, mesangial expansion has a more asymptotic relationship with T1DM duration (Steinke and Mauer, unpublished observations). However, when renal insufficiency occurs, marked mesangial expansion and increased GBM width are present in virtually all T1DM patients.[9,10] Diffuse mesangial expansion, commonly termed *diffuse dia-*

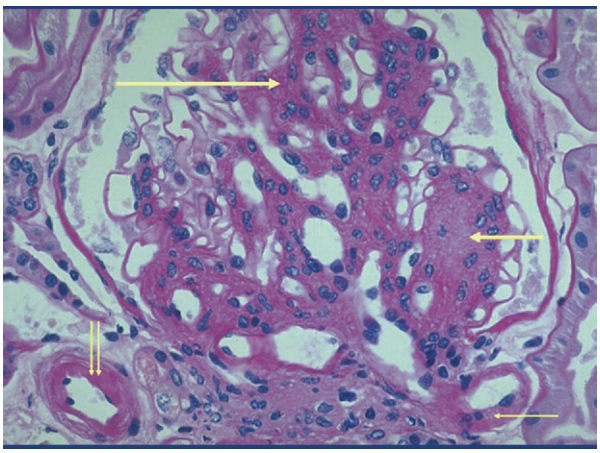

Figure 1. Glomerulus from a T1DM patient with diffuse (long thick arrow) and nodular (short thick arrow) mesangial expansion and afferent (double thin arrows) and efferent (single thin arrow) arteriolar hyalinosis (periodic acid–Schiff [PAS] stain).

betic glomerulosclerosis, can be associated with nodular lesions consisting of areas of marked mesangial expansion forming large round fibrillar mesangial zones with palisading of mesangial nuclei around the periphery of the nodule and compression of the associated glomerular capillaries (Kimmelstiel-Wilson nodules) (Figs. 1 and 2). Both mesangial expansion and GBM and TBM thickening are a consequence of extracellular matrix accumulation, with increased deposition of the normal extracellular matrix local components of types IV and VI collagen, laminin, and fibronectin[12,13] as a result of their increased production, decreased degradation, or both. In contrast to the mesangium, initial interstitial expansion is caused primarily by an increase in the cellular component of this renal compartment[14]; an increase in fibrillar collagen is, in fact, a relatively late finding in this disease, measurable only in patients with an already established decrease in glomerular filtration rate (GFR).[14]

Afferent and efferent arteriolar hyalinosis (Fig. 1) may be present within a few years after diabetes onset.[5] This exudative lesion, which is composed of plasma proteins, especially immunoglobulins, complement, fibrinogen, and albumin, ultimately may replace the smooth muscle cells. The severity of arteriolar hyalinosis is correlated significantly with the percentage of sclerosed glomeruli,[5] suggesting that this vascular lesion could contribute to schemic global glomerular sclerosis. Similar lesions may occur of unknown clinical significance in the glomerular subendothelial space (hyaline caps) and along the parietal surface of Bowman's capsule (capsular drops) (Figs. 3C and 3D).

Abnormalities of the glomerular-tubular junction (GTJA) as late manifestations of the disease,[15] predominantly in patients with proteinuria,[16] with focal adhesions, obstruction of the proximal tubular take-off from the glomerulus, detachment of the tubule from the glomerulus (atubular glomerulus). We classified the GTJA as normal tubules, atrophic tubules (AT), or atubular glomeruli when no tubular connection was observed. AT were subcategorized as short ATs with atrophy of the first few proximal tubular cells, long ATs with atrophy over a longer segment of proximal tubule, and ATs with no observable glomerular opening where no connection between glomerular urinary space and proximal tubular lumen could be discerned (Fig. 3). Atubular glomeruli have open circulation, but with no tubular attachment they presumably are nonfunctioning. GTJA often were associated with tip lesions (ie, focal segmental glomerulosclerosis [FSGS] lesions at or near the GTJ) (Figs. 3A, 3B, and 3C).[15,16] Tip lesions were present in all short ATs, 64% of long ATs, 82% of ATs with no observable glomerular

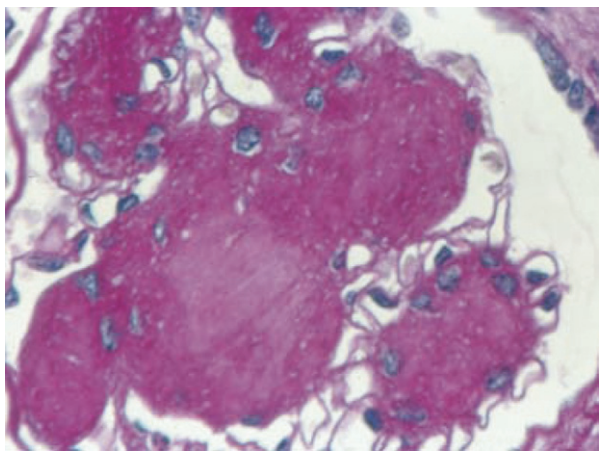

Figure 2. Glomerulus from a T1DM patient with nodular (Kimmelstiel-Wilson) lesions. Note the palisading of nuclei at the periphery of the nodules, the central matrix accumulation, and the restriction of the surrounding glomerular capillaries (PAS). Reprinted with permission from Parving et al.[78]

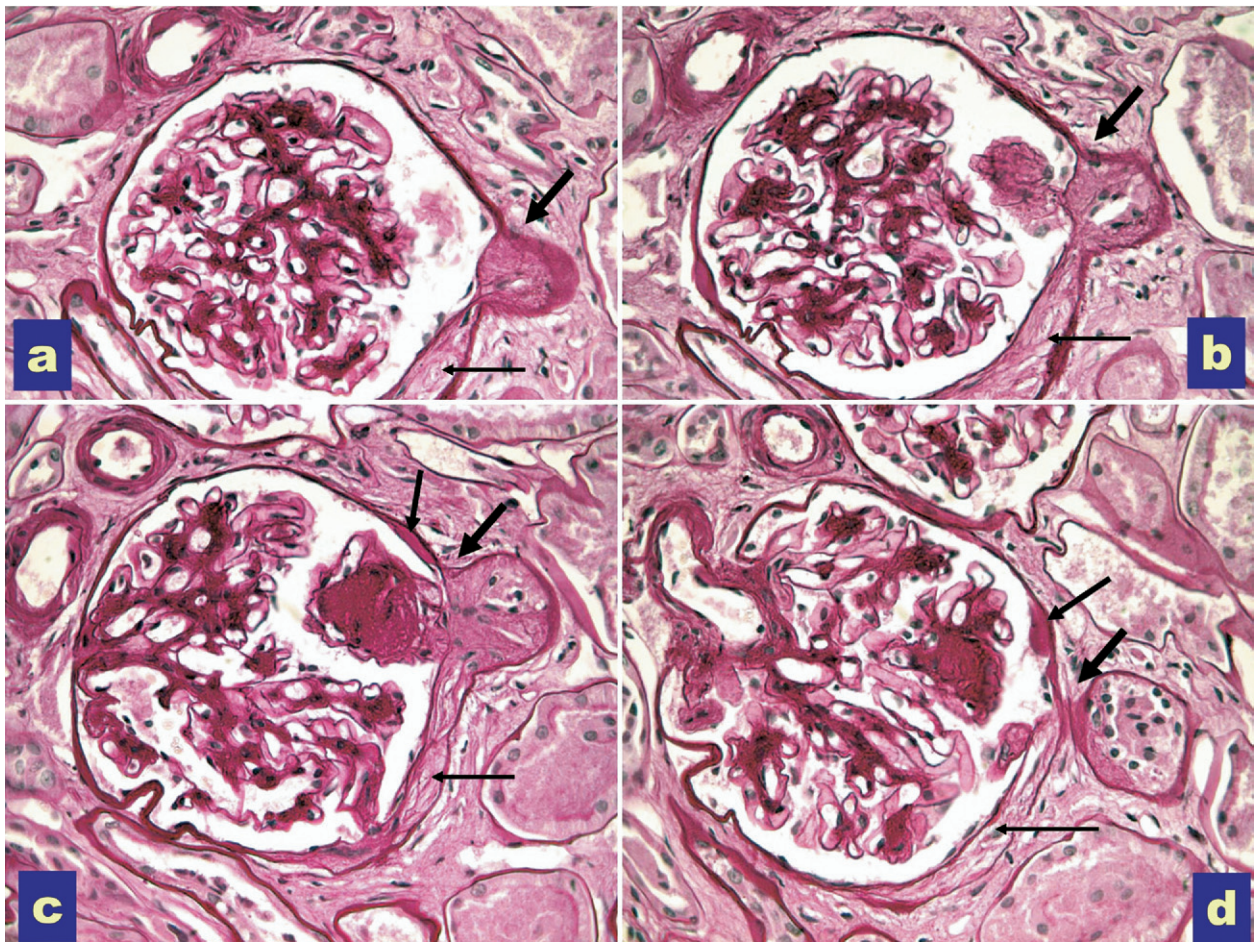

Figure 3. (A, B, C, D) Serial sections through a glomerulus with an abnormal glomerular tubular junction with no observable glomerular opening into the proximal tubule (thick arrows). Note the associated Bowman's capsule abnormalities (thin arrows) and the adhesion of a nodular lesion at the GTJ. (C, D) A capsular drop (short thin arrows) also is present (PAS). Reprinted with permission from Najafian et al.[15]

opening, and 9% of normal tubules, however, they were never observed in normal subjects. GTJA were restricted almost entirely to proteinuric patients, being rare in normoalbuminuric (NA) or microalbuminuric (MA) patients.[16] Moreover, FSGS lesions, also rare in NA and MA patients, had a marked predilection for the GTJ and were uncommon at other locations. Interestingly, the lesions at the GTJ were correlated inversely with GFR[15,16] and thus should be added to the constellation of lesions contributing to the loss of renal function in T1DM.

These various lesions of diabetic nephropathology progress at varying rates within and between T1DM patients, and, as discussed later, this is even more the case in type 2 diabetes mellitus (T2DM). For example, GBM width and mesangial fractional volume [Vv(Mes/glom)], are correlated significantly but not very precisely with one another, with some patients having relatively marked GBM thickening without much mesangial expansion and others the opposite.[9] Marked renal extracellular basement membrane accumulation resulting in extreme mesangial expansion and GBM thickening are present in the vast majority of T1DM patients who develop overt diabetic nephropathy (DN) manifesting as proteinuria, hypertension, and decreasing GFR.[8,17] Ultimately, focal and global glomerulosclerosis, tubular atrophy, interstitial expansion, and GTJA facilitate this downward spiral. However, tubulointerstitial lesions and GTJA contribute only approximately 10% to 15% to functional loss in T1DM

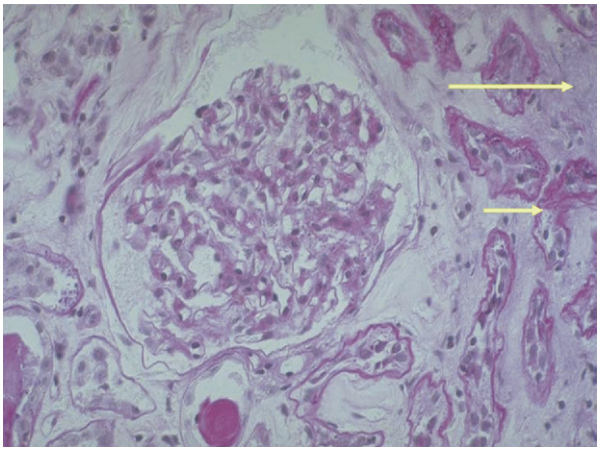

Figure 4. Renal biopsy specimen from a T2DM patient with mild mesangial expansion relative to the severity of interstitial fibrosis (long arrow) and tubular atrophy (short arrow). This would be classified as category III (PAS). Reprinted with kind permission of Springer Science and Business Media.[25]

patients whose GFR is greater than 40 mL/min/1.73m^2.[16] Tubulointerstitial disease may be more important in the progression from moderate renal insufficiency to end-stage renal disease (ESRD),[18] but it is probably a mistake to extrapolate this to earlier stages of DN progression.

The situation in T2DM is more complex. Parving et al[19] described a high prevalence of nondiabetic glomerular diseases in Danish T2DM patients with proteinuria undergoing clinical renal biopsy examinations; 23% had a variety of glomerulopathies including "minimal lesion nephropathy," chronic glomerulonephritis, and mesangial proliferative glomerulonephritis alone or superimposed on diabetic structural abnormalities. Lipkin[20] found that only 50 of 82 T2DM diabetic patients with nephropathy had typical diabetic glomerulopathy; similarly, Gambara et al[21] found that 33% of proteinuric T2DM patients had glomerular disease superimposed on diabetic glomerulosclerosis, although more recently the same group reported that nondiabetic glomerulopathies occurred in only 18% of T2DM patients with proteinuria.[22] In the study by Olsen and Mogensen,[23] only 12% of the proteinuric patients had nondiabetic renal diseases. Thus, the real frequency of nondiabetic renal diseases among patients with T2DM and proteinuria is difficult to assess in studies in which patients underwent a biopsy examination for clinical reasons because of selection bias toward atypical cases; the broad variability described previously likely is related to the different biopsy examination policies adopted, as recently discussed by Mazzucco et al.[24]

We performed renal function studies and research renal biopsy examinations in a large cohort of T2DM patients with MA and proteinuria and described marked heterogeneity in renal structure among these patients; in fact, only a minority had DN patterns typical of those seen in T1DM patients, the remaining patients had mild or absent diabetic glomerulopathy with (Figs. 4 and 5) or without tubulointerstitial, arteriolar, and global glomerulosclerosis changes.[25] Less than 10% of our proteinuric patients had nondiabetic renal diseases. Based on these observations, we proposed a classification system that included 3 major categories.[25] (1) Category C I: normal or near-normal renal structure. These patients (35% of MA and 15% of proteinuric) had normal renal biopsy specimens or showed very mild glomerular, tubular, interstitial, and/or vascular changes. (2) Category C II: typical diabetic nephropathology. These patients (30% of MA and 50% of proteinuric) had established diabetic lesions with an approximately balanced severity of glo-

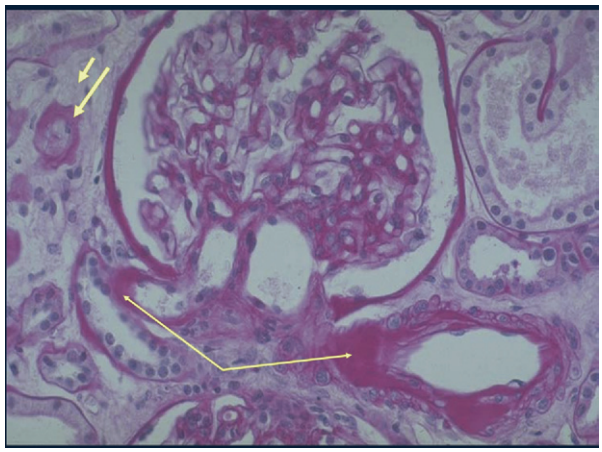

Figure 5. Renal biopsy specimen from a T2DM patient with hyalinosis of the afferent (right thin arrow) and efferent (left thin arrow) glomerular arterioles, interstitial expansion (short thick arrow) and tubular atrophy (long thick arrow). This would be classified as category III (PAS). Reprinted with kind permission of Springer Science and Business Media.[25]

merular, tubulointerstitial, and arteriolar changes, a picture typical of that seen in most T1DM patients with obvious light microscopic DN changes. (3) Category C III: atypical patterns of renal injury (Figs. 4 and 5). These patients (35% of MA and proteinuric) had relatively mild diabetic glomerular changes considered disproportionately severe; category C IIIa: tubular atrophy, TBM thickening and reduplication and interstitial fibrosis (tubulointerstitial lesions); category C IIIb: advanced arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels; and category C IIIc: global glomerular sclerosis. In the category C III group these patterns were present in all possible combinations. More recently, examining the associations of albumin excretion rates (AER) and electron microscopic morphometrically quantitated DN lesions, we could mathematically define a spatial cluster of structural/functional relationships that contained the T1DM patients. About one third of the T2DM patients were categorized outside of this cluster because of MA or proteinuria, despite a paucity of diabetic glomerulopathy lesions.[26] These objective data largely confirm the more subjective categoric classification.

Thus, hyperglycemia may cause different patterns of renal injury in T2DM compared with T1DM patients. Alternatively, the disproportionate tubulointerstitial, glomerulosclerotic, and vascular changes of T2DM also could be related to aging, atherosclerosis, and systemic hypertension. However, it also is possible that the T2DM heterogeneity in renal structure might reflect the heterogeneous nature of T2DM. The natural history of MA and proteinuria in T2DM patients with minimal or no renal lesions is not yet well understood, however, GFR loss in the relatively short term (≈4 y) largely is confined to T2DM research patients with diabetic glomerulopathy.[27]

MORPHOMETRIC ANALYSIS AND STRUCTURAL-FUNCTIONAL RELATIONSHIPS

The relationships between structural abnormalities and kidney function are best defined using light and electron microscopic morphometric analysis. The critical lesion in T1DM is mesangial expansion, morphometrically termed *mesangial fractional volume* (Vv [Mes/glom]) (the fraction of the cross-sectional area of the glomerular tuft made up by mesangium); this is the electron microscopically estimated structural parameter that best correlates with all functional parameters in T1DM.[9,17] Indeed, a highly significant inverse correlation exists between Vv(Mes/glom) and GFR[9,15-17]; when mesangium expands it restricts and distorts glomerular capillaries and diminishes capillary filtration surface,[9] which is strongly directly related to Vv(Mes/glom) and inversely to GFR.[28] Vv(Mes/glom) also is related to AER[9,15-17,29] and blood pressure levels.[30] In contrast, GBM thickening is related closely to AER and less so to GFR or hypertension, suggesting that this lesion is a closer surrogate to the pathogenesis of albuminuria. Interstitial expansion and percentage of global sclerosis also are related directly to proteinuria, hypertension, and inversely to GFR.[4,5,9,15,16] However, our studies in a small number of T1DM patients studied with sequential renal biopsy examinations about 5 years apart indicated that progression from NA to MA and from MA to proteinuria was related primarily to progressive mesangial expansion.[11] In contrast, there was no significant progression in interstitial fibrosis or GBM thickening over this time period. These data initially may seem contradictory to recent studies that described that greater GBM width at baseline biopsy examination was predictive of AER after 5 or 6 years of follow-up evaluation.[31,32] However, given the linear course of GBM thickening versus the nonlinear trajectory of mesangial expansion, it is not surprising that GBM width, a strong correlate of AER, is a better predictor of DN risk whereas mesangial expansion, through its intimate relationship with filtration surface, better defines the clinical course of those destined to develop severe diabetic kidney disease.

Although an increase in AER to the MA range usually is considered the first clinical expression of DN, some long-term T1DM patients have reduced GFR as the initial indicator of renal disease.[33] Thus, T1DM patients, most often females with diabetic retinopathy and/or hypertension, still may be NA despite a reduced GFR. These patients have significantly more ad-

vanced diabetic glomerulopathy lesions than the NA patients with a normal GFR.[33] This situation of "normoalbuminuria with renal dysfunction" also has been seen in T2DM patients[34]; in this study 39% of NA T2DM patients, a majority of whom were women, had an estimated GFR of less than 60 mL/min.[34]

As alluded to previously, through much of the natural history of DN lesions develop in complete clinical silence. When persistent MA and proteinuria supervene, lesions are often far advanced and loss of GFR then may progress relatively rapidly toward ESRD. This typical clinical story is best described by nonlinear analyses of structural-functional relationships.[16] By using simple linear regression models, glomerular structural variables explained about 65% of AER and 35% of GFR variability among T1DM patients.[17] However, using piecewise (spline) regression models, glomerular structural variables alone, GBM width (Vv[Mes/glom], and total filtration surface per glomerulus), explained 95% of the variability in AER ranging from NA to proteinuria. These same glomerular structures, however, explained only 78% of the GFR variability in this study, and this increased to 92% with the addition of indices of GTJA and interstitial expansion.[16]

In summary, most of the AER and GFR changes in T1DM are explained by diabetic glomerulopathy lesions and these structural-functional relationships are driven largely by patients with more advanced lesions and clinical functional abnormalities whereas structure is highly variable (from virtually none to moderate severity) in patients without functional abnormalities. In the end, as in other slowly progressive renal diseases, clinical findings in DN may, at least in part, reflect the lesions exceeding renal compensatory capacities and this may be mirrored in the nonlinear analyses described previously.

Our findings in T2DM confirm that mesangial expansion is a crucial structural change leading to loss of renal function in diabetes. AER is related directly to both GBM width ($r = 0.47$) and Vv(Mes/glom) ($r = 0.44$), but GFR is related inversely only to Vv(Mes/glom) ($r = 0.47$) (Fioretto, unpublished data). Although these structural-functional relationships are highly statistically significant, they are less precise than in T1DM. Moreover, confirming our observations from light microscopic studies,[25,26] glomerular lesions are less advanced in T1DM than T2DM patients and a substantial number of T2DM patients have normal glomerular structure despite an abnormal AER. These data are in agreement with those in Pima Indians, in whom global glomerular sclerosis, interstitial expansion and GBM width were similar in patients with long-term T2DM with NA and those with MA; only Vv(Mes/glom) increased from early diabetes to MA.[35] Ultrastructural glomerular parameters in these patients were significantly abnormal only in patients with clinical nephropathy.[35] Although Hayashi et al[36] reported renal structural-functional relationships in T2DM patients similar to those observed in T1DM, Østerby et al[37] described great variability in glomerular injury in Danish T2DM patients with proteinuria; they also concluded that T2DM patients often had less marked glomerular changes than T1DM patients with comparable renal function. As already noted previously, our cluster analysis studies argue that this greater structural-functional heterogeneity represents increased AER in a substantial subset of T2DM patients that is, at least in part, unexplained by the classic DN lesions.[26] The prognostic relevance of increased AER in this subset of T2DM patients has not yet been described fully.

Nonetheless, the heterogeneity in renal structure is related to the risk of progressive GFR loss.[27] Indeed, GFR decrease over 4 years of follow-up evaluation in T2DM diabetic patients with MA and proteinuria was correlated significantly with the severity of mesangial expansion and GBM thickening.[27] These findings may, in part, explain why treatment trials have shown less dramatic benefits in proteinuric T2DM[38,39] than T1DM[40] subjects. Thus, greater randomization of substantial numbers of proteinuric subjects who are slow progressors or nonprogressors to both treatment arms would blunt a study's ability to show treatment effects. This could be mitigated somewhat by the exclusion at study entry or through subset analyses of T2DM patients without diabetic retinopathy because these subjects are more likely to

have increases in AER levels disproportionate to their diabetic glomerular lesions.[25]

ROLE OF PODOCYTES IN DN

The glomerular filtration barrier is composed of fenestrated endothelium, GBM, podocyte foot processes, and slit diaphragms. Compromise of one or more elements of this filtration complex leads to proteinuria.[41] Podocyte detachment from GBM, from apoptosis, necrosis, or loss of adhesive interaction may play a central role in the pathogenesis of several proteinuric diseases; in fact proteinuria in glomerular disorders ultimately is associated with foot process effacement, flattening, and retraction,[42] although these changes may not be present at the initiation of the glomerular barrier alteration or injury.[41] Podocytes are injured in numerous experimental and human glomerular disorders, including minimal-change disease, FSGS, collapsing glomerulopathy, lupus nephritis, and DN.[42,43]

The understanding of the role of podocytes in DN has increased in recent years, although definitive conclusions have not been reached. Although it is well known that podocyte foot process width increases and slit pore length per GBM surface area decreases with increasing urinary protein excretion in diabetes,[44-46] recent studies have documented that podocyte shape changes, albeit subtle, already are present in NA young T1DM subjects,[47] perhaps consistent with an early role for this cell in the pathogenesis of diabetic glomerulopathy. This may not have been detectable in earlier studies[45] because of confounding of the normal control group with cadaver kidney donor material, which may evidence increased foot process width compared with biopsy specimens obtained in situ from normal living kidney donors (Torbjornsdotter et al[47] and Mauer, unpublished data). Podocyte detachment from GBM, which also may be an early phenomenon in patients with T1DM, worsens with increasing albuminuria[44] and could be responsible for podocyte loss and decreased podocyte number (see later).

White et al[48] observed similar numbers of podocytes in normal subjects and in T1DM patients with abnormal AER, although there was a trend toward fewer podocytes per glomerulus in the diabetic patients. Moreover, this study found no statistically significant correlation between podocyte number and AER. These findings are in contrast to those in earlier T1DM studies[49] that reported decreased podocyte number in patients with normal AER when compared with normal controls, thus suggesting that diabetes per se may adversely affect podocyte reproduction, survival, or both. Low podocyte number also has been described in proteinuric T2DM Pima Indian patients.[35] The investigators'[50] hypothesis that podocyte loss and increased foot process width could play a role in the progression to overt nephropathy was supported in their subsequent longitudinal study in which T2DM MA Pima Indian subjects were studied over 4 years; a greater reduction in the number of podocytes per glomerulus at baseline in these MA subjects predicted a higher risk of progression to overt nephropathy. This variable was a slightly stronger predictor than mesangial fractional volume in these subjects, although were a head-to-head comparison of these 2 variables performed, it is likely that these would be statistically similarly powerful predictors.[50] MA and proteinuric Caucasian T2DM patients that we studied also had decreased length density of filtration slits (FSLv/glom), and increased foot process width over the peripheral GBM compared with NA subjects.[51] AER was related inversely to podocyte numeric density (Nv[epi/glom]) and FSLv/glom and directly to foot process width, although there was no statistically significant correlation with podocyte number per glomerulus (Epi N/glom). GFR was correlated weakly to FSLv/glom, but not to any other podocyte variable. Several patients with abnormal AER had normal Vv(Mes/glom) ($\leq$0.25). We compared their podocyte structure with that of NA patients who also had normal Vv(Mes/glom). Patients with abnormal AER had lower Nv(epi/glom) and FSLv/glom and greater foot process width than NA patients with the similar Vv(Mes/glom). Thus, changes in podocyte structure and density occur at the early stages of DN in Caucasian T2DM patients and might contribute to increasing albuminuria in these patients. Moreover, podocyte structural changes could in part explain or result from increased albuminuria in patients without classic diabetic glomerulopathy.

Podocytes probably have limited capacity to replicate. Podocyte loss, along with the increase in glomerular volume that may occur in diabetes, would require the residual podocytes to cover a larger area of GBM. This might facilitate podocyte detachment, resulting in bare GBM areas with consequent proteinuria. Moreover, these areas of detachment could initiate adhesions and potential starting points for GTJA and focal or global glomerular sclerosis.

RISK FACTORS FOR DN

Although it is clear that genetic factors modulate DN risk and that some patients escape this complication despite decades of poor glycemic control, it also is clear that hyperglycemia is a necessary precondition for DN lesions and renal functional disturbances to develop. Important support for this concept derives from research kidney biopsy studies in identical twins discordant for T1DM. The kidneys of all of the nondiabetic members of these twin pairs were normal structurally, and in each instance GBM and mesangial measures were greater in the diabetic twin of the pair.[52] Several diabetic twins had values for GBM width and Vv(Mes/glom) that were still within the range of normal and thus, the diabetic had changes only in comparison with their nondiabetic twin, whereas others had more severe lesions.[52] Thus, given sufficient duration, probably all T1DM patients have structural changes that are similar in their direction but vary markedly between individuals in the rate at which these lesions develop. The marked variability in the rate of development of lesions of DN in transplanted kidneys also not explained fully by glycemia.[53] Because all the recipients had ESRD secondary to DN in their native kidneys, the absence of detectable disease recurrence in some renal allografts is consistent with intrinsic renal tissue susceptibility to the diabetic state, which may be determined genetically.

Variability in glomerular volume and number also could be structural determinants of nephropathy risk. The mean glomerular volumes were lower in patients developing DN after 15 years of T1DM compared with a group that developed nephropathy after at least 25 years.[54] Thus, glomerular volume increases as mesangial expansion develops and patients unable to respond to mesangial expansion with glomerular enlargement more quickly may lose filtration surface and develop overt nephropathy than those whose glomeruli enlarge. The number of glomeruli per kidney can vary markedly among normal individuals and among diabetic patients[55] and it has been suggested that fewer glomeruli per kidney could be a risk factor for the development of DN.[56] However, T1DM transplant recipients, having a single kidney, do not have accelerated development of diabetic renal lesions compared with T1DM patients with 2 native kidneys (Chang et al, unpublished data). Although of unproven importance in the genesis of DN, reduced glomerular number could result in more rapid progression to ESRD once advanced lesions and overt DN had developed.

Several studies have shown that DN risk clusters in families. Seaquist et al[57] observed that DN occurred in only 2 of the 12 T1DM diabetic siblings of the probands free of DN, whereas 82% of siblings of probands with DN had evidence of diabetic renal disease. These findings have been largely confirmed both in T1DM and T2DM patients.[58-61]

We studied glomerular structure in T1DM sibling pairs and described that the concordance of nephropathy among these pairs was based on concordance in glomerular lesions.[62] In fact, the strongest predictor of glomerular structure in the diabetic sibling was glomerular structure in the diabetic proband.[61] Not only was the severity of individual glomerular lesions highly correlated, but also the patterns of glomerular lesions were similar; for example, if one sibling had a relatively greater increase in GBM width compared with mesangial matrix fractional volume [Vv(MM/glom)], the other sibling was more likely to have the same pattern.[62] In the same cohort, Na/H antiport activity in cultured skin fibroblasts, known to be associated with DN risk,[63] was correlated strongly among sibling pairs.[64] These data support a strong genetic basis for DN, pathogenesis and risk.

Several candidate genes for DN risk or protection have been proposed including genes involved in glucose metabolism, extracellular matrix synthesis, and degradation the renin-angiotensin system and other pathways.[65] Poly-

morphism of genes related to the renin-angiotensin system have been suggested in many studies to be involved in conferring susceptibility for both onset and progression of DN; the D allele of angiotensin-converting enzyme-1 is associated with DN in both T1DM and T2DM patients.[66] We studied the relationships between the insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene and diabetic glomerulopathy in a large group of T2DM patients with abnormal AER.[67] Although renal function was not significantly different among patients with the II, ID, or DD genotype, diabetic glomerulopathy was more severe in DD patients. Moreover, subdivided in tertiles of increasing values of GBM width and mesangial matrix fractional volume, the DD carriers had an odds ratio of 6.11 (confidence interval, 1.84-20.3) and 10.67 (confidence interval, 2.51-45.36) of being in the highest versus the lowest tertile for GBM width and mesangial matrix fractional volume.[67] Thus, among patients with diabetes and abnormal AER, the presence of the DD genotype is associated with a high risk for the development of advanced diabetic glomerulopathy lesions.[67]

Also, we analyzed the relationships between glomerular structure and polymorphism of PC-1 K121Q, a gene associated with insulin resistance, in T2DM patients with MA or proteinuria.[68] The degree of glomerulopathy was similar among XQ and KK patients. Moreover, despite that patients carrying the Q PC-1 genotype had a lower GFR, the decrease of kidney function was similar among the 2 groups, suggesting that this polymorphism is involved in the regulation of renal hemodynamics rather than in the development of glomerular structural abnormalities.[68]

The inconsistent results obtained to date with the candidate gene approach[65] may in large part be owing to the genetic complexities of DN risk. Multiple genes probably are involved in conferring susceptibility to and/or protection from diabetic renal disease and it is important to take into account not only gene-gene interactions, but also the relationships between genetic and environmental factors. Among environmental factors, other than metabolic control, there is increasing interest in smoking as a risk factor for DN. Smoking is an independent risk factor for the development of MA, the rate of progression from MA to proteinuria, and subsequent renal failure both in T1DM and T2DM diabetes.[69] We found that smokers had higher values for AER, GFR, and GBM width than nonsmokers.[70] The increase in GBM width among smokers was consistent with a dose-dependent effect.[70]

REVERSIBILITY OF DN LESIONS

Pancreas transplantation offers the opportunity to test the effects of long-term normoglycemia to prevent, halt, or reverse DN lesions. Pancreas transplantation, performed at the time of renal transplant or within a few years after kidney transplant, prevents or slows the development of early diabetic glomerulopathy lesions in the renal allograft.[71-73]

The possibility of diabetic renal lesion reversal was addressed by long-term studies of the native kidneys of T1DM recipients of pancreas transplantation alone (PTA). We found that despite 5 years of normoglycemia there was no amelioration of the lesions of DN in 13 PTA recipients.[74] GBM width, abnormal before PTA, was unchanged after 5 years. Vv(Mes/glom) was increased compared with baseline because of a decrease in glomerular volume, whereas the total mesangial volume per glomerulus remained unchanged.[74] Eight of the 13 PTA recipients were available for studies after 10 years of normoglycemia.[75] These patients were (mean $\pm$ SD) 33 $\pm$ 3 years old, had diabetes for 22 $\pm$ 5 years, and hemoglobin A_1c of 8.7% $\pm$ 1.5% at the time of PTA. GFR was decreased significantly (by $\approx$25%) at 5 years after PTA but did not change thereafter. AER was decreased at 10 years in those patients with increased baseline values.[75] These renal functional data, however, are difficult to interpret given the confounding effects of cyclosporine.[76] Indeed, there was a significant correlation between the changes in GFR and cyclosporine dose and blood levels in these patients in the first year after transplant.[76] Unlike the 5 year post-PTA results, obvious reversal of diabetic glomerular and tubular lesions was observed in all 8 patients 10 years after PTA.[75] Thus, GBM and TBM widths, unchanged at 5 years, decreased at the 10-year follow-up

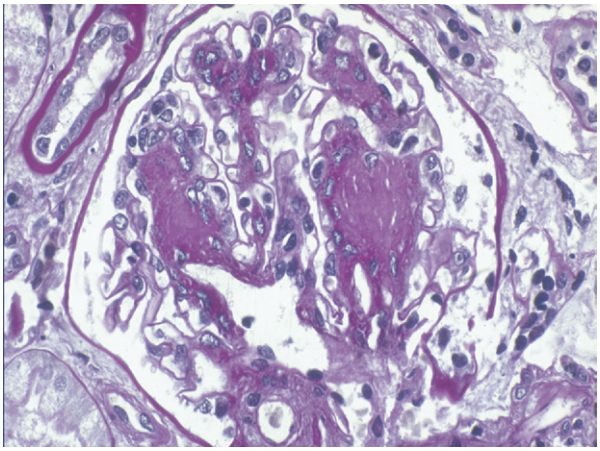

Figure 6. Diffuse and nodular mesangial expansion in a T1DM patient before PTA (PAS). Reprinted with permission from Fioretto et al.[75] Copyright © 1998 Massachusetts Medical Society. All rights reserved.

evaluation, returning to normal values in most patients. Vv(Mes/glom) and mesangial matrix fractional volume (Vv[MM/glom]), which had increased from baseline to 5 years, were lower at 10 years than at baseline or 5 years. The glomerular volume decreased from baseline to 5 years and was stable thereafter. The total mesangial and total mesangial matrix volumes per glomerulus consequently were unchanged at 5 years and markedly decreased at 10 years. Light microscopic observations revealed a remarkable amelioration of glomerular structure in these patients, including the total disappearance of Kimmelstiel-

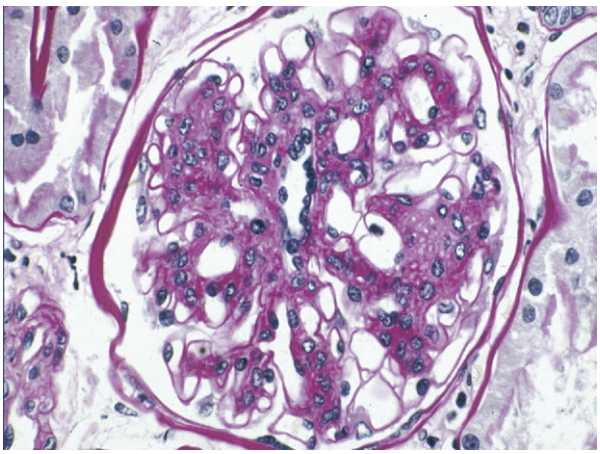

Figure 7. Persistence of diffuse and nodular mesangial expansion 5 years after successful PTA in the same patient as shown in Figure 6. Reprinted with permission from Fioretto et al.[75] Copyright © 1998 Massachusetts Medical Society. All rights reserved.

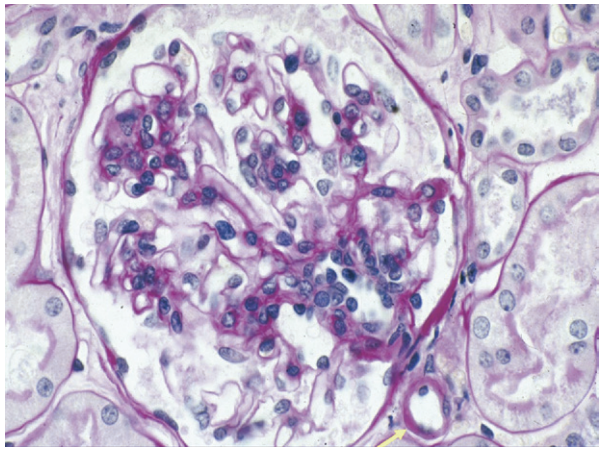

Figure 8. Marked reduction in mesangial expansion 10 years after successful PTA in the same patient as shown in Figures 6 and 7. Note the persistence (arrow) of arteriolar hyalinosis, a common finding in these cases (PAS). Reprinted with permission from Fioretto et al.[75] Copyright © 1998 Massachusetts Medical Society. All rights reserved.

Wilson nodular lesions and the reopening of glomerular capillaries previously compressed by mesangial expansion (Figs. 6-8).[75] The reasons for the long delay in reversal of DN lesions are unknown; nevertheless, the long time necessary for these diabetic lesions to disappear is consistent with their slow development.

More recently we reported remodeling of interstitial and tubular lesions in these same patients.[76] The worsening of interstitial fibrosis and tubular atrophy observed at 5 years post-PTA (Figs. 9,10), likely consequent to cyclo-

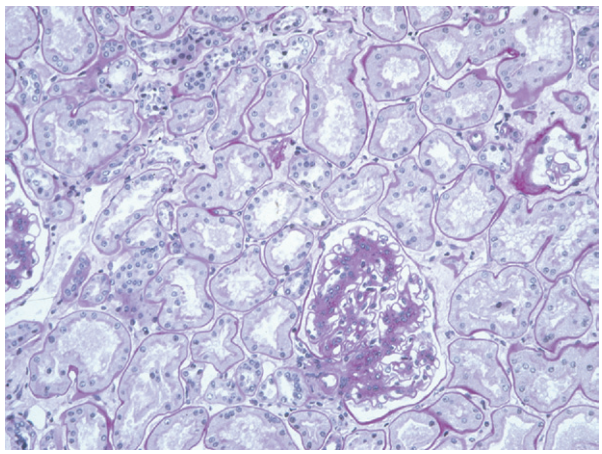

Figure 9. Moderate to advanced diabetic glomerulopathy despite near-normal tubules and interstitium in this T1DM patient before PTA (PAS).

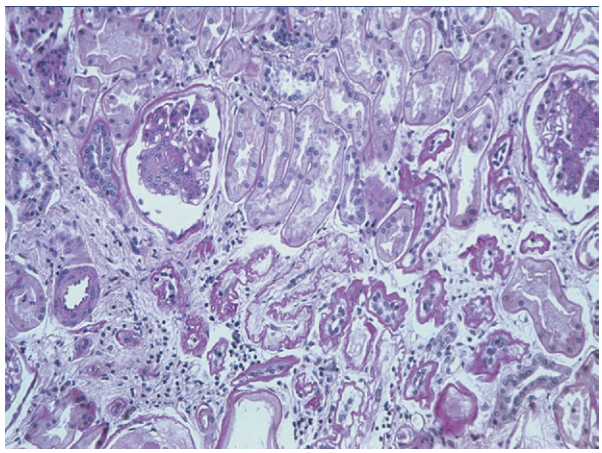

Figure 10. Persistence of the diabetic glomerulopathy changes and de novo interstitial fibrosis and tubular atrophy 5 years after PTA in the same patient as shown in Figure 9 (PAS). Reprinted with permission from Fioretto et al.[76]

sporine therapy,[77] reversed at 10 years after PTA (Fig. 11).[76] We documented remodeling of the tubulointerstitial lesions, with a decrease of cortical interstitial fractional volume, the fractional volume of tubules that were atrophic, and an overall decrease in renal interstitial fibrillar collagen (Figs. 10-11).[76] Whether these structural improvements were consequent to prolonged normoglycemia, decreased cyclosporine dose, or both, was unknown. Regardless of the mechanisms involved, at some point after PTA the glomerular, tubular, and interstitial cells changed their behavior toward extracellular matrix removal and architectural remodeling in remarkable demonstrations of the recovery capacity of the kidney. These findings call for further studies aimed at identifying the molecular and cellular mechanisms involved in these healing processes, which could provide new directions in the treatment of DN.

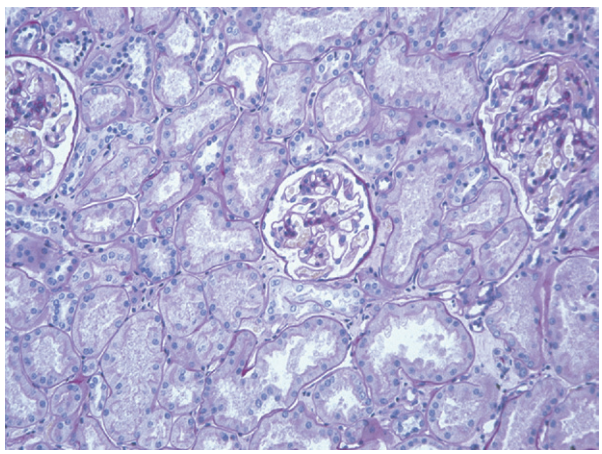

Figure 11. Near-normalization of glomerular structure, marked resolution of interstitial fibrosis, and presumed resorbtion of atrophic tubules 10 years after PTA in the same patient as shown in Figures 9 and 10.

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