Chapter 8
Pathology of the Kidney in Diabetes

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Introduction

In a classical paper published in 1936, Kimmelstiel and Wilson for the first time described mesangial expansion and nodular glomerulosclerosis in diabetic kidney disease (DKD) [1]. We have since learned much more about DKD lesions, although natural history of progression of these lesions is better known in type 1 diabetes, while majority of patients with DKD suffer from type 2 diabetes, calling for further studies in the latter population. The pathology of DKD is also more homogeneous in type 1 diabetes, and there is some controversy if all kidney lesions observed in type 2 are attributable to diabetes or they may be related to concurrent conditions such as aging, hypertension, and atherosclerosis which are commonly present in type 2 diabetic patients. For these reasons, we will initially discuss pathology of DKD in type 1 diabetic patients and then provide comparisons with type 2 diabetes.

DKD in Type 1 Diabetic Patients

The most characteristic features of DKD occur in the glomeruli and include thickening of glomerular basement membrane (GBM), accumulation of mesangial matrix, and arteriolar hyalinosis, which typically occurs in both afferent and efferent arterioles. Evolution of these changes has a well-defined sequence. Using morphometric methods, thickening of GBM is identifiable within 2 years of onset of diabetes. There is a direct and linear relationship between thickness of GBM and duration of

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diabetes [2]. In early stages of DKD, when the patients are normoalbuminuric, there is substantial overlap between GBM thickness in persons with diabetes and nondiabetic individuals which in part may be related to inter-individual variability in the rate of progression of basement membrane thickening or in baseline values of GBM thickness, which is typically unknown [3]. In a study of identical twins who were discordant for type 1 diabetes, all diabetic siblings had thicker GBM and greater mesangial expansion, estimated by the fraction of the volume of glomerulus occupied by mesangium [Vv(Mes/glom)] compared with their nondiabetic siblings. Of note, some of the values for the diabetic subjects were within the "normal range" [4]. Thus, assuming that identical twins had similar GBM thickness and Vv(Mes/glom) values at the onset of diabetes, without a knowledge about the baseline values, these changes would have not been appreciated in diabetic siblings. Age and gender should be taken into account when interpreting GBM thickness values. Ramage et al. showed that GBM thickness in nondiabetic children increases with age, from an average of about 190 nm at 1 year to 300 nm at 11 years, with a reduced rate of increase after the age of 11 years [5], and no difference between males and females in pediatric population. In adulthood, GBM becomes thicker in nondiabetic males averaging about 370 nm in nondiabetic males and 325 nm in females with slight increase in thickness observed up to the fourth decade of life and some decline afterward [6]. In comparison, in normoalbuminuric young type 1 diabetic patients with an average age of 17 years and 8 years of diabetes, the average GBM thickness was 428 nm with a direct relationship with diabetes duration which was not affected by gender [7]. In an older group of type 1 diabetic patients with an average age of about 38 years and about 25 years of diabetes, the average GBM thickness ranged from 465 nm in normoalbuminuric to 700 nm in macroalbuminuric patients [3], where only rare microalbuminuric and virtually no macroalbuminuric patients showed GBM thickness values within the normal range. When examined by transmission electron microscopy, GBM is composed of three distinct components, namely, the lamina rara externa (immediately underneath the foot processes), lamina densa (in the middle and more electron dense), and lamina rara interna (subendothelial). Thickening of GBM in DKD is primarily due to expansion of lamina densa and occurs in a diffuse and uniform fashion. However, especially in advanced DKD, rare glomerular capillaries with thin GBM can be seen. This phenomenon is hypothesized to result from new capillary formation. The hallmark of DKD is accumulation of extracellular matrix, either in the form of thickening of basement membranes or accumulation of mesangial matrix (Fig. 8.1) [8, 9]. This accumulation is related to an imbalance between synthesis, controlled by transcription and translation, and degradation of matrix components, regulated by the interplay between matrix metalloproteinases and their inhibitors [10]. GBM thickening is associated with increased densities of α_3 and α_4 chains of type IV collagen, as hyperglycemia increases production of these molecules by podocytes [11–13].

The earliest lesion of DKD which is appreciable by light microscopy, especially by periodic acid-Schiff stain, is mesangial expansion. Using morphometry, increased Vv(Mes/glom) can be detected as early as 4–5 years after the onset of diabetes [14]. In contrast to thickening of GBM which is more or less linear with increasing dia-

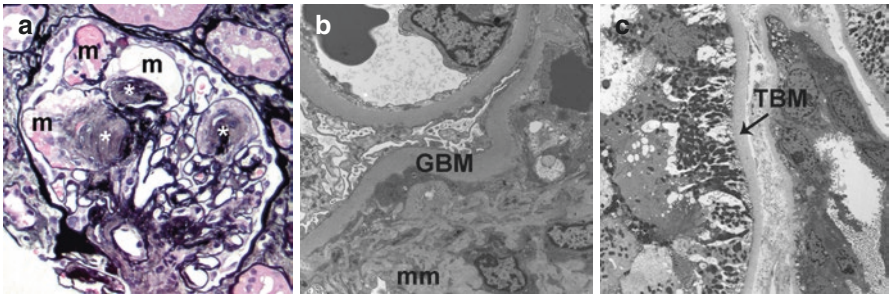

Fig. 8.1 Classical biopsy findings in DKD. (**a**) A glomerulus with nodular glomerulosclerosis or Kimmelstiel-Wilson nodules (*asterisks*) and mesangial expansion due predominantly to increased mesangial matrix and microaneurysm formation (m); Jones methenamine silver stain. (**b**) Thickening of glomerular basement membrane (GBM) and increased mesangial matrix (mm); transmission electron microscopy. (**c**) Thickening of tubular basement membranes (TBM); transmission electron microscopy

betes duration, progression of mesangial expansion is slower in the first few years after the onset of diabetes and becomes faster with increased duration of diabetes [15]. Expansion of mesangium in DKD is due primarily to increased mesangial matrix [7]. Even in the earlier stages when Vv(Mes/glom) is still within the normal range, the fraction of mesangium which is matrix [Vv(MM/glom)], as opposed to mesangial cells, is increased compared to nondiabetic subjects [15]. As mesangium expands, it protrudes into peripheral capillary walls within the subendothelial space, the so-called mesangial interposition. This leads to reduced filtration surface area. Thus, an inverse relationship exists between Vv(Mes/glom) and peripheral GBM filtration surface density [Sv(PGBM/glom)] (Fig. 8.2) [8, 16]. On the other hand, this reduction in Sv(PGBM/glom) is at least partially compensated by increased glomerular volume, preserving the total filtration surface area. Mesangial expansion can be diffuse or nodular. Fraying of the mesangial matrix leads to unfolding of the GBM, conjoining of adjacent capillary loops, and formation of microaneurysms or nodular glomerulosclerosis (so-called Kimmelstiel-Wilson nodules). The accumulated mesangial matrix in the nodules is hypocellular and may show a distinctive lamellated appearance which is best appreciated by Jones methenamine silver stain. The nodules are often surrounded by patent glomerular capillaries or microaneurysms. The microaneurysms may become sclerotic, creating large scarred nodules. Although nodular lesions typically occur in advanced DKD and at least 15 years after the onset of T1D [17, 18], occasional nodular lesions can be seen in earlier stages of DKD when the overall mesangial expansion is mild and diffuse. Therefore, in contrast to the classification proposed by Tervaert et al., the presence of nodular glomerulosclerosis does not always indicate severe DKD. It is noteworthy that nodular glomerulosclerosis is not pathognomonic to DKD and can also be seen in other conditions, perhaps again as a consequence of mesangiolysis, such as light chain deposition disease, immune complex processes, idiopathic nodular glomerulosclerosis, and chronic thrombotic microangiopathy [19].

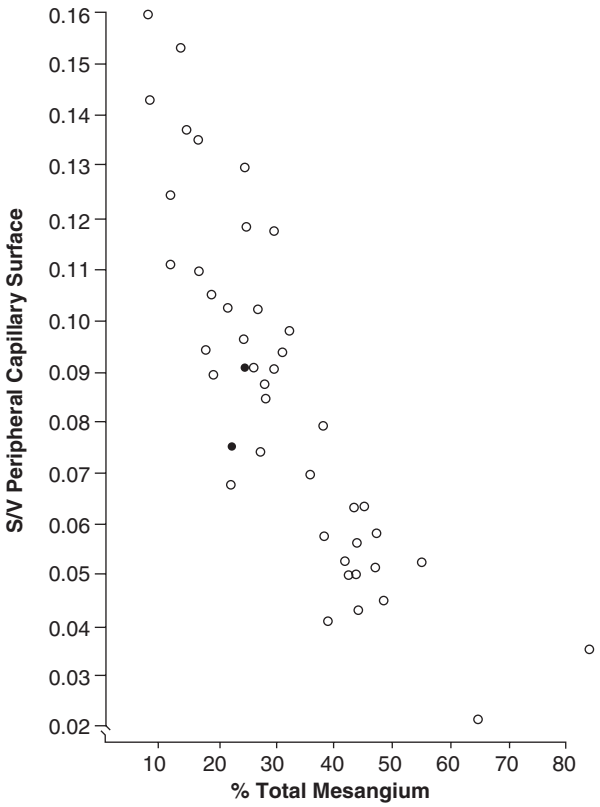

Fig. 8.2 Relationship of percentage total mesangium and S/V of the peripheral capillary surface. $r = -0.86$, $P < 0.0005$. (Figure reproduced from Ref. [8] with permission of the publisher)

Another group of characteristic lesions of DKD result from accumulation of hyaline and are referred to as exudative lesions. These include arteriolar hyalinosis, fibrin caps, and capsular drops (Fig. 8.3). Concomitant hyalinosis of afferent and efferent arterioles is almost specific to DKD and can be seen within 3–5 years after the onset of diabetes [20]. Hyalinosis starts in the subendothelial space but can expand to replace the entire media of arterioles. Some glomeruli may show multiple efferent arterioles at the vascular pole [21]. Fibrin cap, a misnomer which would more appropriately be called "hyaline cap," refers to accumulation of hyaline in glomerular capillary subendothelial spaces. Accumulation of hyaline under parietal epithelial cell lining of Bowman's capsule is called "capsular drop."

While DKD is primarily defined by accumulation of extracellular matrix and its exudative lesions, there is a body of evidence that podocyte injury plays a crucial role in progression of the diseases and kidney prognosis in diabetic patients. About 1/3 of diabetic patients with normal urine albumin excretion rate show increased nephrin excretion in the urine, indicative of early podocyte injury even before the onset of microalbuminuria [22]. Similarly, foot process effacement, commonly regarded as an evidence of podocyte injury, is detectable in normoalbuminuric

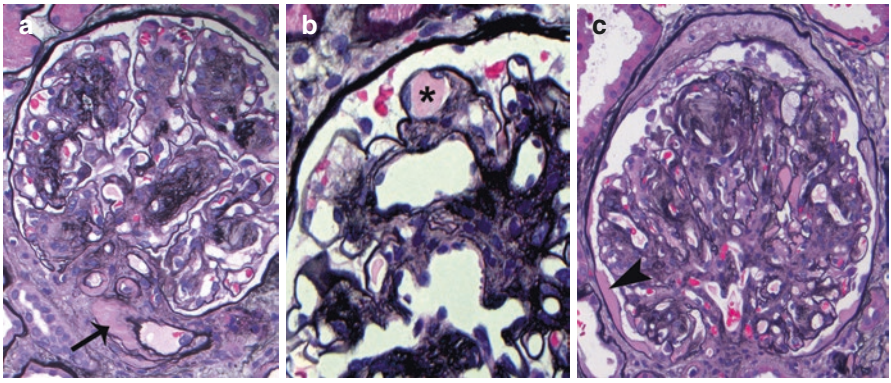

Fig. 8.3 Exudative lesions of DKD. (**a**) Arteriolar hyalinosis (*arrow*). (**b**) Fibrin cap in a glomerular capillary (*asterisk*). (**c**) Capsular drop (*arrowhead*). Jones methenamine silver stain

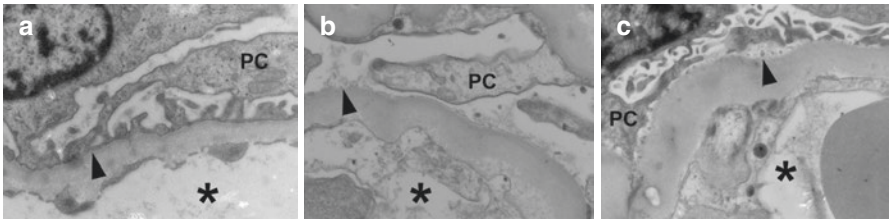

Fig. 8.4 Podocyte (PC)-GBM interfaces (*arrowheads*) are classified into areas with intact foot processes (**a**), areas with no foot process coverage (**b**), and areas with a mixture of intact and detached foot processes (**c**). *Capillary lumen. (Figure reproduced from Ref. [24] with permission of the publisher)

diabetic patients [23] and becomes more severe as albuminuria increases [24]. Various mechanisms are proposed for podocyte injury in diabetes, including reduced expression of $\alpha_3\beta_1$ integrin [25], apoptosis, glucose-induced oxidative stress, and autophagy [26, 27]. Electron microscopy studies show evidence of detachment of podocytes from GBM in normoalbuminuric patients. Similar to foot process effacement, detachment becomes more severe as albuminuria increases (Fig. 8.4) [24]. Podocyte loss and reduced density of podocytes in the glomeruli lead to secondary focal and segmental glomerulosclerosis (FSGS). Notably, FSGS is a relatively late finding in type 1 diabetic patients, when patients are commonly macroalbuminuric. There is a distinct predilection for FSGS lesions to occur at the glomerulotubular junction. A serial section study showed that over half of the FSGS lesions occur at or adjacent to the glomerular tubular outlet, consistent with tip lesion [28, 29]. Thus, it is important to realize that tip lesion is not limited to a subset of primary FSGS and can be seen in various proteinuric conditions, including DKD. A combination of increased shear stress to podocytes at the tubular pole of the glomerular tuft [30] and injury to tubular epithelial cells secondary to the tubulotoxic effect of proteinuria might be involved in predilection of FSGS to this region in conditions with heavy proteinuria [31]. Bowman's capsule thickening and duplication is a common

finding at the FSGS site, perhaps reflecting direction of part of the glomerular ultrafiltrate through into Bowman's capsule, leading to dissection of the capsular basement membrane. This dissection can extend into the glomerulotubular junction, leading to stricture and occlusion of the glomerular tubular outlet and eventually creation of atubular glomeruli (Fig. 8.5), or extend into the proximal tubule [28, 29].

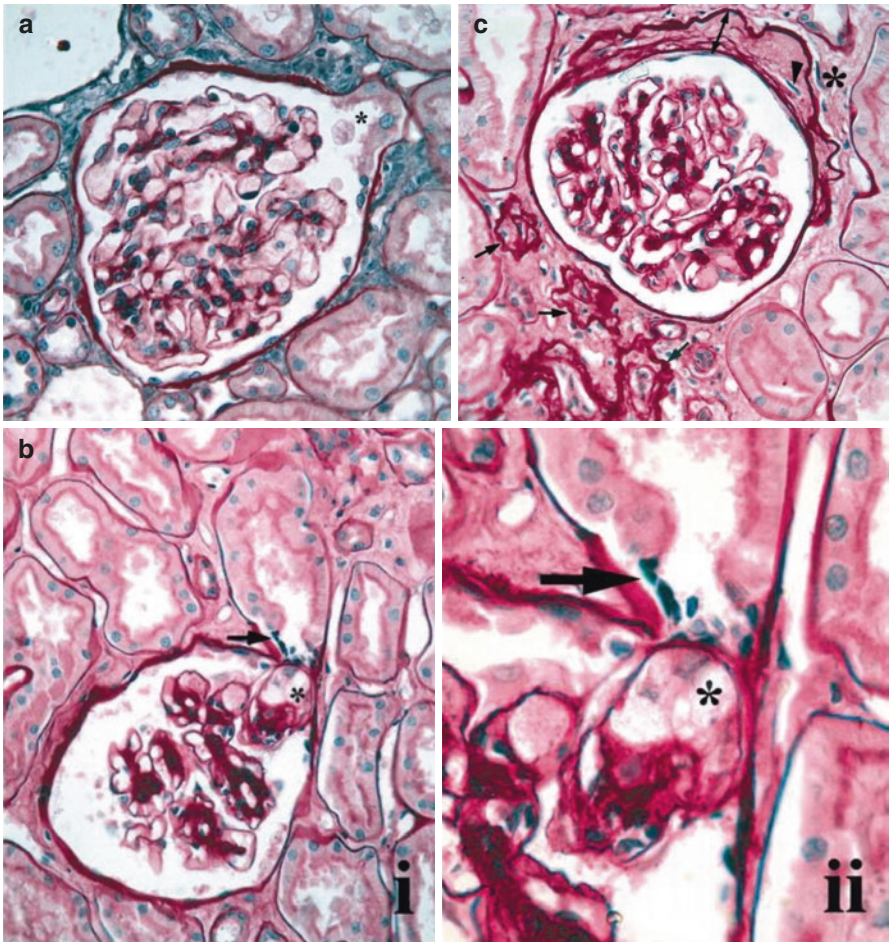

Fig. 8.5 (**a**) A glomerulus attached to a normal tubule (NT). * Glomerulotubular junction. (**b**) (i) A glomerulus attached to a short atrophic tubule (SAT), with a tip lesion at glomerulotubular junction. PAS-stained; magnification, ×630. (ii) A higher-magnification view of the tip lesion, allowing better appreciation of a dilated loop (*), with foam cells within the tip lesion and flat epithelial cells (*arrow*) covering the very beginning of the proximal tubule. (**c**) An atubular glomerulus (AG). The glomerular tuft is indistinguishable from other glomeruli. Bowman's capsule is markedly thickened and wrinkled at a site opposite to the vascular pole, where a tubular connection is expected. ↔, reduplicated Bowman's capsule; arrowhead, a spindle-shape cell within the reduplicated Bowman's capsule; arrow, atrophic tubules adjacent to the atubular glomerulus; * periglomerular fibrosis. PAS-stained; magnification, ×630. (Figure reproduced from Ref. [29] with permission of the publisher)

Thickening of tubular basement membranes (TBM) parallels GBM thickening and is an early finding in DKD (Fig. 8.1) [4, 9]. TBM thickening related to diabetes is diffuse and homogeneous with a different appearance from the nonspecific TBM thickening in atrophic tubules where basements become irregular, corrugated, and duplicated and frequently associated with deposition of cellular debris. Supportive of this difference, TBM width in diabetic patients correlates strongly with GBM width and Vv(Mes/glom) but only weakly with the volume fraction of renal cortex that is interstitium [Vv(Int/cortex)] [9]. Moreover, tubulointerstitial fibrosis follows glomerulopathy in T1D patients. In fact, as a result of tubular hypertrophy, Vv(Int/cortex) initially reduces [32]. Expansion of cortical interstitium is initially due primarily to an increase in the cellular component, while increased interstitial fibrillar collagen deposition occurs relatively late, when GFR decline is already present [32].

Using immunofluorescence microscopy, GBM and TBM commonly show modest linear staining with IgG (polytypical) and albumin in diabetic patients. This finding is related to diabetes, regardless of the presence or absence of DKD. The exact cause of this phenomenon remains unclear, although alterations in chemical properties of extracellular matrix, immunoglobulins, or both might be involved. A recent study suggested an association between the intensity of IgG staining and renal outcomes, but this finding requires further validation, especially given the absence of a proper way to precisely standardize fluorescent intensity of IgG staining [33].

Structural-Functional Relationships of DKD in T1D

The natural history of DKD in T1D patients is characterized by an initial long period of normal or high GFR and normoalbuminuria, during which the disease has a slow progression rate. This initial period is followed by a more rapid pace of increasing albuminuria and GFR loss [34]. The structural-functional relationship models of DKD follow a similar course. Initially, when the patients are normoalbuminuric, classical DKD glomerular structural parameters, including GBM width, Vv(Mes/glom), and Sv(PGBM/glom), may be within the normal range. As progression of DKD leads to microalbuminuria and macroalbuminuria, GBM thickness and Vv(Mes/glom) increase, and Sv(PGBM/glom) reduces. These parameters show considerably less overlap with normal values in microalbuminuric patients and almost no overlap in macroalbuminuric patients [3]. Persistent microalbuminuria is associated with progression of the lesions and increased risk for developing macroalbuminuria [3]. Vv(Mes/glom) and GBM width directly and Sv(PGBM/glom) inversely correlate with urine albumin excretion rate (AER) from normoalbuminuria to macroalbuminuria (Fig. 8.6). Importantly, increased GBM width can predict progression of DKD in T1D patients from normoalbuminuria to microalbuminuria or even to macroalbuminuria and ESRD [35]. In a longitudinal study, none of the normoalbuminuric patients with long-standing T1D and normal GBM width progressed to proteinuria or ESRD after an average follow-up of 11 years [35]. Vv(Mes/glom), fractional volume of mesangial matrix per glomerulus [Vv(MM/glom)], and

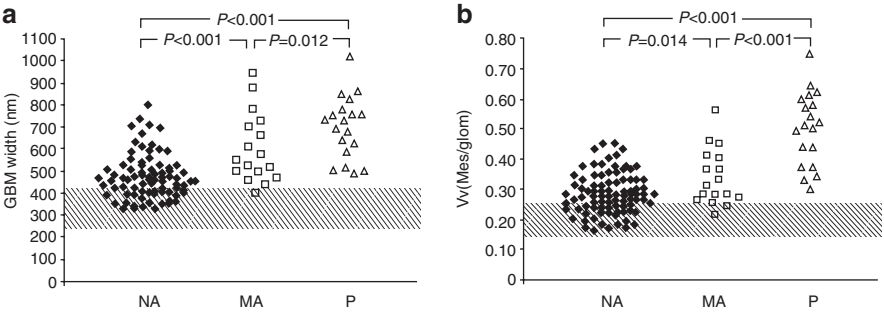

Fig. 8.6 (**a**) GBM width in 88 normoalbuminuric (NA), 17 microalbuminuric (MA), and 19 proteinuric (P) patients with type 1 diabetes. The hatched area represents the mean ± 2 SD in a group of 76 age-matched normal control subjects. All groups are different from control subjects. (**b**) Vv(Mes/glom) in 88 normoalbuminuric (NA), 17 microalbuminuric (MA), and 19 proteinuric (P) patients with type 1 diabetes. The hatched area represents the mean ± 2 SD in a group of 76 age-matched normal control subjects. All groups are different from control subjects. (Figure reproduced from Ref. [3] with permission of the publisher)

GBM width are inversely and Sv(PGBM/glom) is directly related to GFR (Fig. 8.7) [3]. In fact, there is a direct correlation between the total peripheral capillary filtration surface area and GFR from hyperfiltration to renal insufficiency.

T1D patients are fairly homogeneous in regard to DKD structural-functional relationship models, and such models have been shown to be robust [36]. Current models can better predict AER than GFR. About 70% of AER and only about 20–30% of GFR variances are explainable by structural-functional relationship models developed by multiple regression analysis based on glomerular lesions alone. However, models developed by piecewise linear regression analysis can explain much larger fraction of AER and GFR variances, approaching prediction of over 80% of AER and over 65% of GFR variances. Piecewise linear regression analysis examines if the relationships can be explained by two regression lines of different slopes, intersecting at a breakpoint. Thus, the improved predictability of the piecewise linear regression analysis models mirrors the natural history of DKD with an initial slow progression prior to a breakpoint and fast progression thereafter. Importantly, the breakpoints found in two separate studies one based on a small cohort and another based on a larger cohort of T1D patients were both in the microalbuminuric and normal GFR ranges [28, 36], suggesting that the shift from a slow to a fast progression phase occurs relatively early and during the initial clinically silent phase. In addition, these results indicate that glomerular lesions alone can explain a major proportion of AER and GFR variance in T1D patients. In fact, predictability of these models showed relatively minor improvements by adding Vv(Int/cortex) and glomerulotubular junction abnormalities as other predictor variables [28]. In contrast to these results, some studies have suggested that GFR decline in DKD is primarily driven by interstitial fibrosis, rather than diabetic glomerulopathy [37, 38].

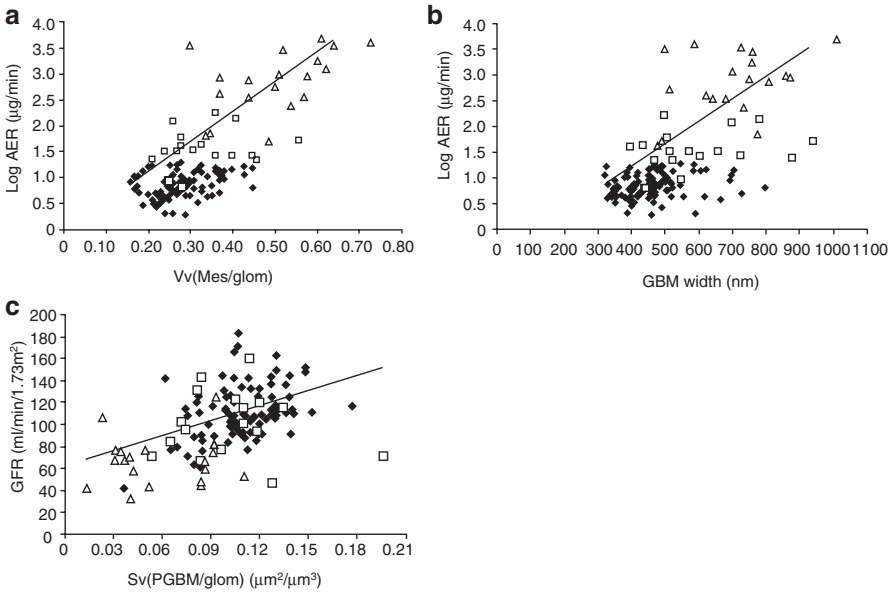

Fig. 8.7 (**a**) Correlation between Vv(Mes/glom) and AER in 124 patients with type 1 diabetes. ♦, Normoalbuminuric patients; ■, microalbuminuric patients; △, proteinuric patients. $r = 0.75$, $P < 0.001$. (**b**) Correlation between GBM width and AER in 124 patients with type 1 diabetes. ◇, Normoalbuminuric patients; ■, microalbuminuric patients; △, proteinuric patients. $r = 0.63$, $P < 0.001$. (**c**) Correlation between Sv(PGBM/glom) and GFR in 125 patients with type 1 diabetes. ◇, Normoalbuminuric patients; ■, microalbuminuric patients; △, proteinuric patients. $r = 0.48$, $P < 0.001$. (Figure reproduced from Ref. [3] with permission of the publisher)

However, as pointed out earlier, increased Vv(Int/cortex) in DKD in T1D patients is seen in later stages when diabetic glomerulopathy becomes more advanced. Moreover, appreciation of contribution of glomerular lesions in GFR loss requires careful measurement of glomerular structural parameters using morphometric techniques. The importance of vascular lesions in advancement of chronic injury in DKD should not be underestimated. An autopsy study showed that the sclerotic glomeruli in T1D patients are more often clustered in the plane vertical to the renal capsule, indicative of the importance of vascular lesions and chronic ischemia in glomerulosclerosis [39].

There is a large body of evidence about podocyte injury and progression of DKD. Foot process width, a parameter that is commonly regarded a sign of podocyte injury, was directly correlated with AER and inversely with GFR across a wide range of albuminuria [24]. Another study showed that the density of podocytes per glomerular volume [Nv(Podo/glom)] was inversely related to AER in normotensive proteinuric T1D patients [40]. Such association was not found between AER and total number of podocytes per glomerulus, perhaps signifying the importance of imbalance between the number of podocytes and glomerular volume as suggested in other glomerulopathies. Of note, relationship between

AER and Nv(Podo/glom) was not seen in microalbuminuric T1D patients. Moreover, another study showed that podocyte structural parameters did not predict progression to proteinuria or ESRD during long-term follow-up in normoalbuminuric T1D patients [36], suggesting that podocyte injury may play a more critical role in progression of DKD in later stages of the disease or, alternatively, the role of podocyte injury may become evident only after a certain fraction of podocytes are lost [41].

The endothelial cells develop structural changes in DKD. There is a reduction in glomerular endothelial fenestration in normoalbuminuric patients which persists in micro- and macroalbuminuria [24]. Although endothelial glycocalyx cannot be observed using routing electron microscopy techniques, it has been shown that DKD is associated with increased heparanase activity that leads to reduced endothelial glycocalyx, a change that can contribute into albuminuria and infiltration of macrophages into the kidney [42].

DKD in Type 2 Diabetic Patients

The frequency of DKD among clinical biopsies, regardless of the status of diabetes, has progressively increased over the last three decades in the USA, currently approaching ~20% [43, 44]. This is while an autopsy study showed that ~19% of diabetic patients with obvious DKD lesions did not present with clinical manifestations of DKD, suggesting that DKD may be underdiagnosed based on indication biopsies [45]. Although type 2 diabetes is by far the most common etiology of ESRD, there are more studies available describing the natural history of DKD lesions in type 1 compared to type 2 diabetes. In general, similarities between DKD lesions in T1D and T2D patients are substantial. Classical glomerular lesions of DKD, including GBM thickening, mesangial expansion, and reduced glomerular filtration surface area, similar to type 1 diabetes, are present and progress with diabetes duration [46]. Studies performed on adults who develop type 2 diabetes later in life when hypertension and atherosclerotic vascular lesions are already present suggest that pathologic findings are more heterogeneous in T2D compared to type 1 diabetic patients [9, 47–49]. Fioretto et al. identified three different patterns or categories of lesions in kidney biopsies from microalbuminuric and macroalbuminuric Northern Italian T2D patients: category I with almost normal biopsies (35% of microalbuminuric and 10% of proteinuric patients) (Fig. 8.8), category II with classical lesions of DKD similar to T1D (30% of microalbuminuric and 55% of proteinuric patients), and category III with disproportionately advanced tubulointerstitial fibrosis, arteriolar hyalinosis, arteriosclerosis, or global glomerulosclerosis, despite minor diabetic glomerulopathy [9]. Of note, these categories correlated with some clinical phenotypes. Thus, the presence of classical DKD lesions (category II) was associated with longer duration of diabetes, poorer glycemic control, faster GFR

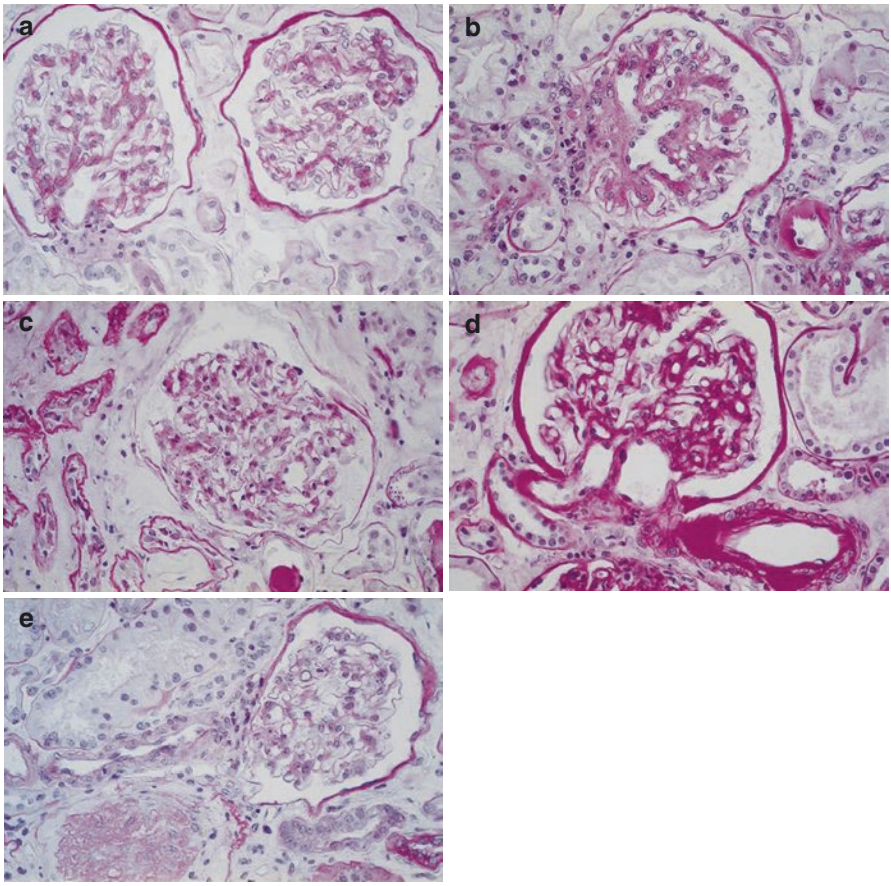

Fig. 8.8 (**a**) Glomeruli from a patient in category C I. Glomerular structure is near normal with minimal mesangial expansion (PAS). (**b**) Glomerulus from a patient in category C II, with well-established diabetic nephropathology. Diffuse mesangial expansion, advanced arteriolar hyalinosis, and mild interstitial fibrosis are present (PAS). (**c**) Glomerulus from a patient in category C III (a), with near-normal glomerular structure and tubular basement membrane thickening, tubular atrophy, and severe interstitial fibrosis (PAS). (**d**) Glomerulus from a patient in category C III (b), with mild mesangial expansion and severe arteriolar hyalinosis, affecting both afferent and efferent glomerular arterioles (PAS). (**e**) Glomeruli from a patient in category C III (c). Glomerular structure is near normal in one glomerulus, while the adjacent shows global glomerular sclerosis (PAS). (Figure reproduced from Ref. [18] with permission of the publisher)

decline, and retinopathy [50, 51]. In contrast, retinopathy was rare in patients exhibiting category I or II on biopsies [52]. In contrast, biopsy studies performed in Pima Indians who generally develop type 2 diabetes at a younger age suggest that the relationships between albuminuria and DKD structural changes are more similar to those seen in type 1 diabetic patients [53], reflecting more homogeneity in DKD lesions in these younger type 2 diabetic patients.

Structural-Functional Relationships in T2D Patients

There is substantial similarity in structural-functional relationships of DKD between type 1 and type 2 diabetes, albeit these relationships may be less precise in type 2 diabetes, which may be at least partly related to the heterogeneity of lesions in older type 2 diabetic patients. A study on 47 Caucasian adults (average age about 60 years) with type 2 diabetes and proteinuria showed direct relationships between Vv(Mes/glom), Vv(MM/glom), and GBM width and proteinuria and inverse relationships between Vv(Mes/glom), Vv(MM/glom), Vv(Int/cortex), and GFR [54]. A longitudinal study in Japanese type 2 diabetic patients found GBM width and Vv(Mes/glom) as predictors of progression of albuminuria after 6 years of follow-up [55]. Another longitudinal study performed on Northern Italian type 2 diabetic patients showed that GBM width and Vv(Mes/glom) predicted GFR decline after follow-up for 4 years [50]. Importantly, even in patients who were normoalbuminuric at the baseline, these lesions predicted GFR loss at follow-up. More recently, a study performed on a large cohort of Pima Indians with type 2 diabetes suggested that both glomerular and tubulointerstitial lesions were significant contributors into GFR loss. On one hand, glomerular parameters, including Vv(Mes/glom), percentage of global glomerular sclerosis, nonpodocyte (mesangial and endothelial) cell number per glomerulus, GBM width, mean glomerular volume, and podocyte foot process width; lower Sv(PGBM/glom); and fewer endothelial fenestrations were each associated with GFR decline after adjustment for main clinical parameters [56]. Moreover, a composite glomerulopathy index, reflecting the combined effects of the statistically significant morphometric variables listed above, was strongly associated with GFR loss. On the other hand, when GFR slope was modeled as a threshold, only Vv(Int/cortex) was associated with the slope. Importantly, these relationships between biopsy structural parameters and GFR loss were even present when the baseline GFR was normal or elevated, suggesting that the deteriorating impact of these lesions on renal function starts at very early stages of DKD when the disease is clinically silent.

Podocyte injury starts early in type 2 diabetic patients. Normoalbuminuric patients with type 2 diabetes show increased urine nephrin and/or podocin mRNA compared to nondiabetic persons [57]. Injured podocytes can detach from the GBM and fall into the urine. In fact, microalbuminuric and proteinuric type 2 diabetic patients show increased shedding of podocytes into the urine (podocyturia) [58]. Since podocytes do not regenerate efficiently, podocyte loss is generally regarded as a cumulative insult to the glomerulus and in time leads to podocyte depletion in the glomeruli. The number of podocytes in a glomerulus can be assessed either in relative (to glomerular volume), i.e., podocyte number density per glomerular volume, or absolute (i.e., podocyte number per glomerulus) terms. It has been shown that both number and number density of podocytes per glomerulus are reduced in microalbuminuric and macroalbuminuric type 2 diabetic patients [53, 59, 60]. Podocyte loss increases with diabetes duration, and as expected this is associated with

increased AER [46, 59]. Once podocyte loss is severe enough, it ensues into segmental and eventually global glomerulosclerosis. Importantly, Meyer et al. found that podocyte number per glomerulus in microalbuminuric Pima Indian persons with type 2 diabetes was not only the strongest predictor of AER increase but also predicted progression to overt nephropathy [61].

Nondiabetic Renal Disease in Diabetic Patients

Currently, indication biopsies in diabetic patients are performed if the clinical history raises suspicion for a nondiabetic renal disease (NDRD), including [62] nephrotic-range proteinuria or kidney failure in the absence of diabetic retinopathy, with diabetes duration less than 5 years or with normal GFR, reduced GFR with diabetes duration less than 5 years, unexplained microscopic hematuria or acute kidney injury, or rapidly worsening kidney function in patients with previously stable kidney function. Therefore, it is not surprising to find a high incidence of NDRD in clinical biopsies from diabetic patients [63–68]. Given the prevalence of diabetes, up to 25% of all clinical renal biopsies are done in diabetic patients [69]. The prevalence of NDRD in biopsies from diabetic patients is variable in the literature and ranges from 10% to 85% [70–73]. However, the incidence of NDRD in research or protocol biopsies is remarkably lower than in clinical biopsies [74]. The likelihood of finding NDRD in indication biopsies from diabetic patients is affected by the criteria and biopsy threshold used, as well as ethnic and geographic factors. In a study performed at Columbia University, of all indication biopsies from diabetic patients, 37% had DKD alone, 36% had NDRD alone, and 27% had DKD plus NDRD [69]. In the NDRD alone group, the most common diagnosis was FSGS (22%), followed by hypertensive nephrosclerosis, acute tubular injury, IgA nephropathy, membranous nephropathy, and pauci-immune glomerulonephritis. In the DKD plus NDRD group, however, acute tubular injury was the most common finding (43%), followed by hypertensive nephrosclerosis, FSGS, and IgA nephropathy. Diabetes duration ≥12 years was found to be the best predictor of DKD alone.

It is noteworthy that some lesions listed among the most commonly reported forms of NDRD in biopsies from diabetic patients, e.g., focal and segmental glomerulosclerosis and hypertensive nephrosclerosis, can reflect processes secondary to DKD, rather than independent concurrent diseases. There is no general consensus on how to report such lesions. Therefore, studies on NDRD made based on extracting data from pathology reports may well be affected by reporting routines of one center vs. another. Another example would be the presence of interstitial eosinophilic aggregates that are commonly regarded as an allergic reaction to presumptive drugs. However, Dai et al. showed that this finding, which can be seen in about 40% of indication biopsies from diabetic patients, does not correlate with a clinical history of drug allergy or the number of medicines used by patients and instead it correlates with the severity of chronic injury in renal parenchyma [75].

DKD Classification of Pathologic Lesions

Classification of pathologic lesions facilitates uniform reporting of biopsy findings and reproducibility of data generated from biopsy studies. Tervaert et al. [76] proposed a pathologic classification for DKD mainly based on the glomerular lesions. The classification consists of four progressive classes, including GBM thickening (class I), mesangial expansion (class II which is divided into classes IIa if mild and IIb if severe), presence of Kimmelstiel-Wilson nodules (class III), and extensive global glomerulosclerosis (class IV). Vascular and tubulointerstitial lesions are included in a separate scoring system. An et al. in a large study performed on type 2 diabetic patients showed that the severity of glomerular and interstitial lesions inversely impacts renal prognosis [77]. Another study showed that progression of glomerular, tubulointerstitial, and vascular lesions evaluated by this classification was associated with poor renal prognosis [33]. On the other hand, some studies have challenged the prognostic significance of glomerular lesions according to this classification [78, 79]. Whether this classification has any predictive value in early stages of DKD, when treatments are more likely to affect outcomes, remains to be validated. In an effort to study the net cumulative effect of various DKD lesions on renal prognosis, Hoshino et al. proposed a D-score calculated by summing the scores of all components in Tervaert classification which led to improvement in prediction of renal outcome, with a D-score ≤ 14 predicting excellent outcomes [80]. However, it should be noted that all studies confirming a prognostic value for this classification so far basically have reported that more severe glomerular or tubulointerstitial lesions portend worse outcomes, which is not surprising and does not help identifying patients at greater risk of progression at earlier stages. In addition, some other important aspects of DKD with predictive value for renal dysfunction, such as heterogeneity of patterns of renal injury in T2D [70], and some other morphologic features with predictive value for renal dysfunction, such as podocyte loss [53], glomerulotubular junction abnormalities [28], or endothelial fenestration [56], are not included in this classification.

Are DKD Lesions Reversible?

It has been shown that kidney lesions developed in diabetic murine models are reversible following normoglycemia. Islet transplantation in STZ-induced diabetic rats normalizes blood glucose and leads to reversal of diabetic kidney lesions in 2 months [81]. BTBR ob/ob diabetic mice, a model of type 2 diabetes with kidney lesions mimicking those seen in human DKD, show reversal of diabetic lesions after 6 weeks of leptin replacement-associated normoglycemia [82]. However, as explained earlier in this chapter, human DKD lesions in contrast to murine models gradually develop in a long time. Similarly, long-term normoglycemia is required for human DKD lesions to improve or reverse. Fioretto et al. showed that after

10 years of normoglycemia following pancreas transplantation, marked reversal of diabetic glomerulopathy lesions can be seen in type 1 diabetic patients with a diabetes duration of approximately 20 years, while 5 years of normoglycemia after pancreas transplantation was not enough to lead to appreciable changes [83]. Most strikingly, Kimmelstiel-Wilson nodules had completely disappeared in the 10-year biopsies. GBM and TBM width, Vv(Mes/glom), and Vv(MM/glom) were all reduced at 10 years compared with the baseline and 5-year values, and these parameters in some patients returned to the normal at 10-year biopsies (Fig. 8.9) [9]. Reversal of diabetic glomerulopathy was also associated with improvement of tubulointerstitial lesions and reduction in total cortical interstitial collagen [28].

Given the limited regeneration capacity of podocytes and the role of podocyte loss in progression of DKD lesions (see above), it is important to find out if podocyte regeneration is needed for reversal of DKD. Animal models have suggested that progenitor cells on Bowman's capsule may be involved in replacing lost podocytes in the glomerular tuft [82, 84–87]. Pichaiwong et al. showed that leptin treatment of BTBR ob/ob mice not only led to reversal of renal diabetes lesions but also was associated with podocyte regeneration in the glomeruli [82]. Evidence as to whether or not that is the case in reversal of DKD lesions in humans is scanty. In one study that addresses this issue, Andeen et al. showed that early DKD in clinical biopsies was associated with increased number of parietal cells with a podocyte phenotype (Fig. 8.10), indicative of the potential for podocyte restoration [19].

The effect of pharmaceutical intervention to reverse DKD or reduce its progression has also been explored in limited studies. Five years of RAAS blockade by losartan or enalapril in normotensive normoalbuminuric type 1 diabetic patients did not prevent progression of DKD lesions but reduced progression of retinopathy [88]. On the other hand, 6 years of treatment with losartan slowed progression of mesangial expansion in microalbuminuric Pima Indian patients with type 2 diabetes [89].

Animal Models of DKD

Animal models have been widely used to explore the pathogenesis of DKD [90, 91]; however, in general, they do not faithfully replicate human DKD. A detailed discussion of models of DKD in several animal species is presented in Chapter 13 of this book. Most murine models show only the earliest features of human DKD, and an ideal model is yet to be developed. In response to this need, the nephropathy subcommittee of the Animal Models of Diabetic Complications Consortium (AMDCC) sets validation criteria for rodent models of DKD based on the clinical and pathological features of human DKD [92], including [1] >50% decrease in renal function, [2] >10-fold increase in albuminuria, and [3] pathological features including advanced mesangial matrix expansion with or without nodules, thickening of the GBM, arteriolar hyalinosis, and tubulointerstitial fibrosis.

Common rodent models used for type 1 diabetes include streptozotocin (STZ)-induced diabetic mice, Akita mice, OVE26 FVB mice, and nonobese diabetic

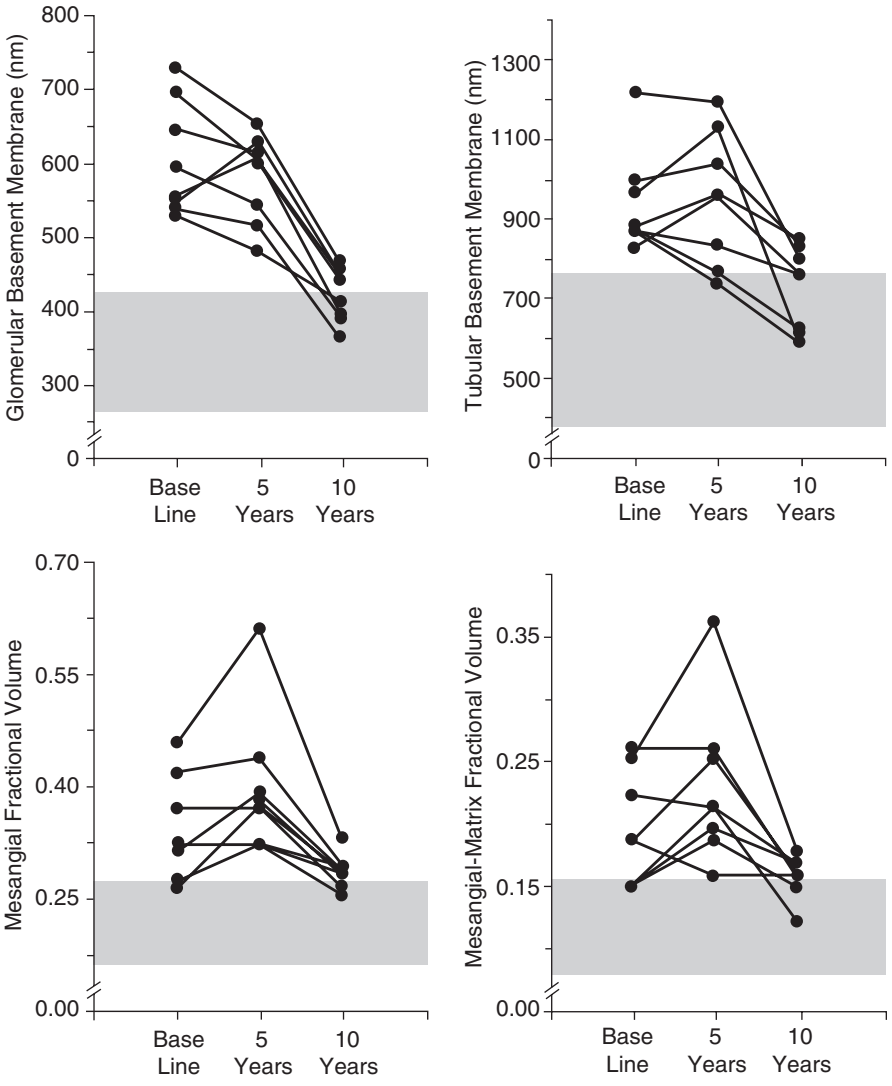

Fig. 8.9 Thickness of the glomerular basement membrane, thickness of the tubular basement membrane, mesangial fractional volume, and mesangial-matrix fractional volume at baseline and 5 and 10 years after pancreas transplantation. The mesangial fractional volume is the proportion of the glomerulus occupied by the mesangium; the mesangial-matrix fractional volume is the proportion of the glomerulus occupied by mesangial matrix. The shaded areas represent the normal ranges obtained in the 66 age- and sex-matched normal controls (means ±2 SD). Data for individual patients are connected by lines. (Figure reproduced from Ref. [9] with permission of the publisher)

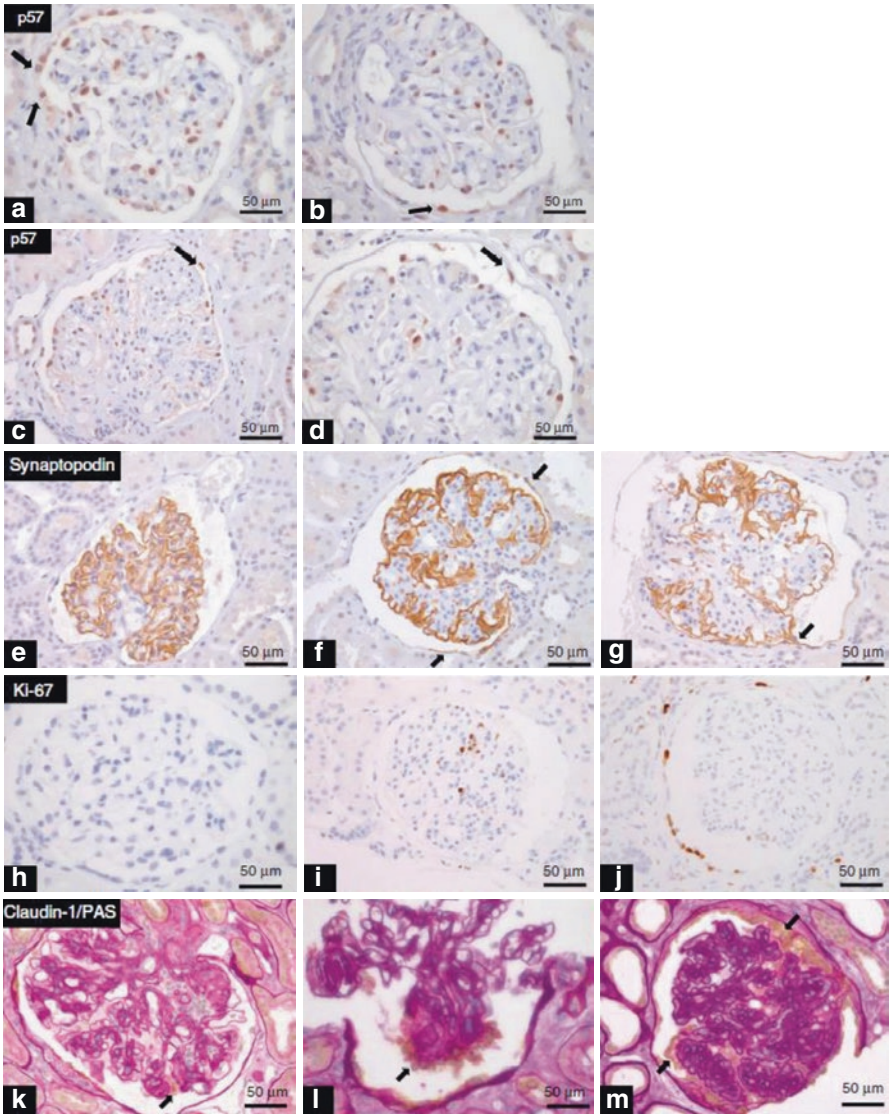

Fig. 8.10 Immunophenotypic alterations in podocyte and parietal epithelial cells in diabetic nephropathy (DN). (**a**–**d**) Cells marking as podocytes were present in parietal epithelial cell locations and significantly increased in histologically early DN (**a**, **b**), with a nonsignificant increase in advanced DN (**c**, **d**) compared with controls (original magnification ×400). (**e**–**g**) Synaptopodin highlighted a significantly increasing percentage of staining of cells lining Bowman's capsules from controls (**e**) to early (**f**) to advanced DN, including areas of segmental adhesions (original magnification ×400). (**h**–**j**) Ki-67-expressing cells were identified on the glomerular tuft and Bowman's capsule in morphologically early (**i**) and advanced (**j**) DN but only rarely in controls (**h**) (original magnification ×400). (**k**–**m**) Claudin-1/PAS revealed claudin-1-positive cells in areas of increased mesangial matrix in early DN (**k**), in areas of "capping" of segmentally sclerotic regions (**l**), and having a variable glomerular distribution in advanced DN (**m**) (original magnification ×400). (Figure reproduced from Ref. [19] with permission of the publisher)

(NOD) mice. STZ is a chemical toxin for pancreatic β cells. Therefore, injection of sufficient dose of STZ can make virtually any pre-existing model diabetic, although it should be noted that susceptibility to develop diabetic DKD varies among different strains [92]. For example, C57BL/6J mice in general are resistant to the development of kidney injury, including DKD [92]. It should be noted that STZ, especially if used at high doses (150–200 mg/kg), is nephrotoxic [93]. Multiple injections of low doses can avoid this problem to some degree [94]. Diabetic C57BL/6J mice develop mesangial expansion and some thickening of GBM, but not nodular glomerulosclerosis or tubulointerstitial fibrosis [92]. STZ-induced diabetes has been tried on other mouse strains such as DBA/2, CD1, and 129/Sv and also in rats [95]. Multiple genetic models for type 1 diabetes have also been developed. Akita mice have an *Ins2+/C96Y* mutation (a single nucleotide substitution in the *Ins2* gene) [96], which leads to abnormal folding of the insulin protein with subsequent toxic injury to pancreatic β cells and development of diabetes. It has been shown that the genetic background of Akita mutation mice affects the severity of albuminuria and histological changes. Although *Ins2+/C96Y* mutation causes comparable hyperglycemia in C57BL/6, DBA/2, and 129/SvEv mice, the DBA/2-*Ins2+/C96Y* mice develop more severe albuminuria, but C57BL/6 and 129/SvEv mice develop more prominent increase in mesangial matrix [97]. However, Akita mice, regardless of the background strain, do not develop advanced DKD lesions, such as mesangiolysis, nodular glomerulosclerosis, or tubulointerstitial fibrosis. Thus, these mice can be considered for modeling early to moderate DKD [95]. Moreover, C57BL/6-*Ins2+/C96Y* mice develop diffuse granular mesangial IgA deposits starting at 20 weeks of age, which is a confounding factor for analysis of the contribution of diabetes to the mesangial injury that may develop [98]. Another model of type 1 diabetes is the OVE26 FVB mice with transgenic overexpression of calmodulin in pancreatic β cells with subsequent deficiency in insulin production within the first week of life [99]. This model develops progressive albuminuria, starting by 2 months of age. GFR increases from 2 to 3 months of age, followed by a subsequent decline from 5 to 9 months, with increased systolic and diastolic blood pressures. Diabetic OVE26 FVB mice develop glomerulomegaly, GBM thickening, podocyte loss, mesangial matrix increase, nodular glomerulosclerosis, and tubulointerstitial fibrosis [100]. Therefore, this model exhibits some of the features of advanced DKD in humans. The nonobese diabetic (NOD) mouse, which develops type 1 diabetes through autoimmune destruction of islet cells, is similar to humans [101, 102]. However, this model faces some disadvantages to others including the complex genetic background required for development of disease, the inconsistent timeline for onset of hyperglycemia, and the development of autoimmunity including deposition of immune complexes in glomeruli [103]. Perhaps for the same reasons, the extent of diabetic kidney injury in NOD mice has not been well characterized.

The most common type 2 diabetes murine models include db/db mice, KK-Ay mice, T2DN/Mcwi mice, eNOS−/− *db/db* mice, *OVE26–TTrhRen* double trans-

genic mice, BTBR *ob/*ob mice, Zucker diabetic fatty (ZDF) rats, Wistar fatty rats, Otsuka Long-Evans Tokushima fatty (OLETF) rats, and Goto-Kakizaki (GK) rats.

db/db mice have a deletion mutation in the leptin receptor (*LepRdb/db*) which causes abnormal splicing and results in a defective receptor for the adipocyte-derived hormone leptin [104]. Defected leptin signaling leads to abnormal hypothalamic responses, ensuing in hyperphagia, obesity, hyperlipidemia, hyperinsulinemia, insulin resistance, and diabetes, which is more severe in male mice than in females. Male *db/db* mice become hyperglycemic at 6–10 weeks of age, followed by moderate to severe albuminuria at 8–25 weeks of age. Renal function declines at 15–18 weeks. db/db mice develop GBM thickening, podocyte loss, and moderate mesangial matrix expansion, but not mesangiolysis, nodular glomerulosclerosis, or severe tubulointerstitial fibrosis [26, 105]. KK mice develop mild insulin resistance and obesity, which is more severe in male animals [106, 107]. KK mice develop mild increase in mesangial matrix and GBM thickening. However, STZ-induced diabetic KK/H1J mice show more severe mesangial matrix expansion with nodular glomerulosclerosis and arteriolar hyalinosis [108]. The KK-*Ay* mouse was developed by transferring the yellow obese gene (*Ay* allele) into the KK mouse, which then becomes severely obese, hyperglycemic, and albuminuric. The kidneys of these mice show diffuse and moderate to severe mesangial matrix expansion with mesangial cell proliferation, segmental glomerulosclerosis, nodular glomerulosclerosis, and podocyte loss [109, 110]. The Zucker fatty (ZF) rat has a homozygous missense mutation (*fatty, fa*) in the leptin receptor gene (*Lepr*), resulting in obesity without diabetes. Zucker diabetic fatty (ZDF) rats are derived from the ZF strain. These rats are obese and develop progressive insulin resistance and diabetes [111, 112]. They are not hypertensive and show an initial increase in GFR which later on declines to normal level. Pathological changes include glomerulosclerosis, tubulointerstitial fibrosis, and inflammation [113]. The Wistar fatty (WF) rat is a congenic strain of the Wistar Kyoto (WKY) rat with a *fa/fa* homozygous missense mutation in the *Lepr* gene, resulting in obesity, hyperinsulinemia, and hyperlipidemia [114, 115]. Diabetes in the WF rats is milder than in the ZDF rats. However, the WF rats develop GBM thickening, foot process effacement, mesangial expansion, and tubulointerstitial inflammation. The Otsuka Long-Evans Tokushima fatty (OLETF) rat is a robust model of type 2 diabetes. Almost all male OLETF rats develop diabetes by 25 weeks of age [116]. These rats develop albuminuria, proteinuria, and elevated GFR. Long-lasting diabetes in the OLETF rats is associated with glomerulomegaly, increased mesangial matrix, GBM thickening, nodular glomerulosclerosis, and tubulointerstitial fibrosis [117]. The Goto-Kakizaki (GK) rat is a nonobese model of type 2 diabetes, developed from a colony of Wistar rats through selection of rats with hyperglycemia [118]. The GK rats demonstrate impaired glucose tolerance test as early as 2 weeks of age, due to hypoplasia of pancreatic islet cells and insulin resistance [119]. GK rats develop type 2 diabetes by 12 weeks of age. However,

they are relatively resistant to develop DKD [120], although some levels of GBM thickening and mild to moderate mesangial expansion have been reported in this model [121]. T2DN/Mcwi mice which are developed from a cross between GK and fawn-hooded hypertensive (FHH) rats [122] develop diabetes and progressive proteinuria, focal glomerulosclerosis, severe mesangial matrix expansion, and GBM thickening and later on nodular glomerulosclerosis and arteriolar hyalinosis [123].

Additional genetic stressors have been incorporated into some of the genetic models of DKD to accelerate progression of the lesions. The full knockout of endothelial nitric oxide synthase (eNOS) on the *db/db* C57BL/KsJ background results in eNOS−/− *db/db* mice which are hypertensive and develop marked albuminuria and reduced GFR with aging, extensive mesangial matrix expansion with nodules, mesangiolysis, increased GBM thickness, arteriolar hyalinosis, and tubulointerstitial fibrosis [124, 125]. Chronic activation of the renin-angiotensin system (RAS) in hyperreninemic transgenic (*TTRhRen*) mice is another approach to accelerate kidney lesions [126]. STZ injection to *TTRhRen* transgenic mice results in albuminuria and kidney lesions. *OVE26–TTrhRen* double transgenic mice develop very prominent albuminuria with glomerulosclerosis and interstitial fibrosis [126]. Combining the black and tan, brachyuric (BTBR) mouse strain with natural insulin resistance, with the *ob/ob* leptin mutation, results in BTBR ob/ob mice [127, 128]. These mice develop hyperglycemia and albuminuria with prominent mesangial matrix expansion, focal nodular glomerulosclerosis, mild GBM thickening and arteriolar hyalinosis and podocyte loss. Importantly, many of these lesions, including podocyte loss, can be reversed by administration of leptin [82]. However, the phenotype reported by some other labs has been milder than what was originally described, perhaps reflecting the impact of environmental factors on DKD in this model [129].

Characteristics of some of the discussed models are tabulated in Table 8.1. In summary, most currently studied mouse models of diabetes show early morphological changes of human DKD, such as mesangial matrix expansion and, in some cases, podocyte loss, including *db/db* and Akita mice. There are few models that exhibit features of both morphologically early and late DKD; of these, the eNOS−/− *db/db* mice, OVE26 FVB mice (a type 1 diabetes model), and BTBR *ob/*ob mice (modeling type 2 diabetes and obesity) appear to be the most robust. The BTBR *ob/*ob mouse model is particularly noteworthy for the relative rapidity in which lesions develop, making it well suited for studies of new therapeutics. Despite the plethora of diabetic mouse models, all models available to date possess important limitations in their practicality and/or fidelity in recapitulating all of the features of human disease. The tubulointerstitial and vascular lesions of DKD have been particularly challenging to model in the mouse. Designing better models of DKD that will allow identification of underlying mechanisms remains an important research objective, which in turn will facilitate testing of therapeutic interventions that can ameliorate or even reverse the structural alterations of DKD.

Table 8.1 Renal functional and pathologic characteristics of various murine models of DKD

Model	Strain	Characteristic								
		Hypertension	GFR decline	Albuminuria	Mesangial expansion	Glomerulosclerosis	Arterial hyalinosis	GBM thickening	IFTA	References
Type 1 diabetes										
STZ	C57BL/6J	–	–	–	+	–	–	+	–	[108, 130]
	DBA/2J	–	–	++	+	+	+	++	–	[108, 130]
Akita (*Ins2+/C96Y*)	C57BL/6J	+	–	+	+	–	NR	NR	–	[97]
	129/SvEv	+	–	++	+	–	NR	NR	–	[97]
	FVB/NJ	+	–	++	++	–	NR	–	–	[131]
OVE26	FVB/NJ	+	–	+++	+++	++	+	++	+	[132, 133]
OVE26—TTRhRen	FVB/NJ	+++	+	+++	+++	NR	–	NR	++	[126]
Type 2 diabetes										
ob/ob	BTBR	–	–	++	+++	++	+	+	+	[82, 128]
	FVB/NJ	–	NR	++	++	+	+	++	+	[134, 135]
	C57BL/KsJ	–	–	+++	++	–	+	+	–	[136]
eNOS−/− *db/db*	C57BL/KsJ	++	++	+++	+++	++	+	++	+	[124]
KK	KK/H1J	NR	–	++	+++	++	+	++	–	[108]
	KK-A(y)/Ta	–	–	+++	+++	++	–	+	–	[110]
ZDF	ZDF/Gmi™	–	–	++	++	+	NR	NR	+	[112, 137]
WFR	fa/fa	NR	+	+++	+	–	NR	+	+	[115]
OLETF	OLETF	+	–	++	++	+	NR	+	+	[117, 138]
GK	T2DN/Mcwi	+	–	++	+++	+	+	+	+	[123]

Modified from Azushima et al. [139]

References

1. Kimmelstiel P, Wilson C. Intercapillary lesions in glomeruli in kidney. Am J Pathol. 1936;12:83–97.
2. Osterby R. Kidney structural abnormalities in early diabetes. Adv Metab Disord. 1973;2(Suppl 2):323–40.
3. Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, et al. Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. Diabetes. 2002;51(2):506–13.
4. Steffes MW, Sutherland DE, Goetz FC, Rich SS, Mauer SM. Studies of kidney and muscle biopsy specimens from identical twins discordant for type I diabetes mellitus. N Engl J Med. 1985;312(20):1282–7.
5. Ramage IJ, Howatson AG, McColl JH, Maxwell H, Murphy AV, Beattie TJ. Glomerular basement membrane thickness in children: a stereologic assessment. Kidney Int. 2002;62(3):895–900.
6. Steffes MW, Barbosa J, Basgen JM, Sutherland DE, Najarian JS, Mauer SM. Quantitative glomerular morphology of the normal human kidney. Lab Invest. 1983;49(1):82–6.
7. Drummond K, Mauer M. The early natural history of nephropathy in type 1 diabetes: II. Early renal structural changes in type 1 diabetes. Diabetes. 2002;51(5):1580–7.
8. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest. 1984;74(4):1143–55.
9. Brito PL, Fioretto P, Drummond K, Kim Y, Steffes MW, Basgen JM, et al. Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. Kidney Int. 1998;53(3):754–61.
10. Mariappan MM. Signaling mechanisms in the regulation of renal matrix metabolism in diabetes. Exp Diabetes Res. 2012;2012:749812.
11. Zhu D, Kim Y, Steffes MW, Groppoli TJ, Butkowski RJ, Mauer SM. Glomerular distribution of type IV collagen in diabetes by high resolution quantitative immunochemistry. Kidney Int. 1994;45(2):425–33.
12. Moriya T, Groppoli TJ, Kim Y, Mauer M. Quantitative immunoelectron microscopy of type VI collagen in glomeruli in type I diabetic patients. Kidney Int. 2001;59(1):317–23.
13. Bai Y, Wang L, Li Y, Liu S, Li J, Wang H, et al. High ambient glucose levels modulates the production of MMP-9 and alpha5(IV) collagen by cultured podocytes. Cell Physiol Biochem. 2006;17(1-2):57–68.
14. osterby R. Early phases in the development of diabetic glomerulopathy. Acta Med Scand Suppl. 1974;574:3–82.
15. Steffes MW, Bilous RW, Sutherland DE, Mauer SM. Cell and matrix components of the glomerular mesangium in type I diabetes. Diabetes. 1992;41(6):679–84.
16. Ellis EN, Steffes MW, Goetz FC, Sutherland DE, Mauer SM. Glomerular filtration surface in type I diabetes mellitus. Kidney Int. 1986;29(4):889–94.
17. DeFronzo RA. Diabetic Nephropathy: Etiologic and therapeutic considerations. Diabetes Review. 1995;3:510–64.
18. Fioretto P, Steffes MW, Mauer M. Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. Diabetes. 1994;43(11):1358–64.
19. Andeen NK, Nguyen TQ, Steegh F, Hudkins KL, Najafian B, Alpers CE. The phenotypes of podocytes and parietal epithelial cells may overlap in diabetic nephropathy. Kidney Int. 2015;88(5):1099–107.
20. Mauer SM, Barbosa J, Vernier RL, Kjellstrand CM, Buselmeier TJ, Simmons RL, et al. Development of diabetic vascular lesions in normal kidneys transplanted into patients with diabetes mellitus. N Engl J Med. 1976;295(17):916–20.
21. Osterby R, Asplund J, Bangstad HJ, Nyberg G, Rudberg S, Viberti GC, et al. Neovascularization at the vascular pole region in diabetic glomerulopathy. Nephrol Dial Transplant. 1999;14(2):348–52.

22. Patari A, Forsblom C, Havana M, Taipale H, Groop PH, Holthofer H. Nephrinuria in diabetic nephropathy of type 1 diabetes. Diabetes. 2003;52(12):2969–74.
23. Perrin NE, Torbjornsdotter TB, Jaremko GA, Berg UB. The course of diabetic glomerulopathy in patients with type I diabetes: a 6-year follow-up with serial biopsies. Kidney Int. 2006;69(4):699–705.
24. Toyoda M, Najafian B, Kim Y, Caramori ML, Mauer M. Podocyte detachment and reduced glomerular capillary endothelial fenestration in human type 1 diabetic nephropathy. Diabetes. 2007;56(8):2155–60.
25. Chen HC, Chen CA, Guh JY, Chang JM, Shin SJ, Lai YH. Altering expression of alpha3beta1 integrin on podocytes of human and rats with diabetes. Life Sci. 2000;67(19):2345–53.
26. Susztak K, Raff AC, Schiffer M, Bottinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes. 2006;55(1):225–33.
27. Anil Kumar P, Welsh GI, Saleem MA, Menon RK. Molecular and cellular events mediating glomerular podocyte dysfunction and depletion in diabetes mellitus. Front Endocrinol (Lausanne). 2014;5:151.
28. Fioretto P, Sutherland DE, Najafian B, Mauer M. Remodeling of renal interstitial and tubular lesions in pancreas transplant recipients. Kidney Int. 2006;69(5):907–12.
29. Najafian B, Kim Y, Crosson JT, Mauer M. Atubular glomeruli and glomerulotubular junction abnormalities in diabetic nephropathy. J Am Soc Nephrol. 2003;14(4):908–17.
30. Friedrich C, Endlich N, Kriz W, Endlich K. Podocytes are sensitive to fluid shear stress in vitro. Am J Physiol Renal Physiol. 2006;291(4):F856–65.
31. Baines RJ, Brunskill NJ. Tubular toxicity of proteinuria. Nat Rev Nephrol. 2011;7(3):177–80.
32. Katz A, Caramori ML, Sisson-Ross S, Groppoli T, Basgen JM, Mauer M. An increase in the cell component of the cortical interstitium antedates interstitial fibrosis in type 1 diabetic patients. Kidney Int. 2002;61(6):2058–66.
33. Mise K, Hoshino J, Ubara Y, Sumida K, Hiramatsu R, Hasegawa E, et al. Renal prognosis a long time after renal biopsy on patients with diabetic nephropathy. Nephrol Dial Transplant. 2014;29(1):109–18.
34. Ponchiardi C, Mauer M, Najafian B. Temporal profile of diabetic nephropathy pathologic changes. Curr Diab Rep. 2013;13(4):592–9.
35. Caramori ML, Parks A, Mauer M. Renal lesions predict progression of diabetic nephropathy in type 1 diabetes. J Am Soc Nephrol. 2013;24(7):1175–81.
36. Harindhanavudhi T, Parks A, Mauer M, Caramori ML. Podocyte structural parameters do not predict progression to diabetic nephropathy in normoalbuminuric type 1 diabetic patients. Am J Nephrol. 2015;41(4-5):277–83.
37. Thomsen OF, Andersen AR, Christiansen JS, Deckert T. Renal changes in long-term type 1 (insulin-dependent) diabetic patients with and without clinical nephropathy: a light microscopic, morphometric study of autopsy material, Diabetologia. 1984;26(5):361–5.
38. Bohle A, Wehrmann M, Bogenschutz O, Batz C, Muller CA, Muller GA. The pathogenesis of chronic renal failure in diabetic nephropathy. Investigation of 488 cases of diabetic glomerulosclerosis. Pathol Res Pract. 1991;187(2-3):251–9.
39. Horlyck A, Gundersen HJ, Osterby R. The cortical distribution pattern of diabetic glomerulopathy. Diabetologia. 1986;29(3):146–50.
40. White KE, Bilous RW, Marshall SM, El Nahas M, Remuzzi G, Piras G, et al. Podocyte number in normotensive type 1 diabetic patients with albuminuria. Diabetes. 2002;51(10):3083–9.
41. Matsusaka T, Sandgren E, Shintani A, Kon V, Pastan I, Fogo AB, et al. Podocyte injury damages other podocytes. J Am Soc Nephrol. 2011;22(7):1275–85.
42. Rabelink TJ, de Zeeuw D. The glycocalyx--linking albuminuria with renal and cardiovascular disease. Nat Rev Nephrol. 2015;11(11):667–76.
43. O'Shaughnessy MM, Hogan SL, Thompson BD, Coppo R, Fogo AB, Jennette JC. Glomerular disease frequencies by race, sex and region: results from the International Kidney Biopsy Survey. Nephrol Dial Transplant. 2017; https://doi.org/10.1093/ndt/gfx189.

44. O'Shaughnessy MM, Hogan SL, Poulton CJ, Falk RJ, Singh HK, Nickeleit V, et al. Temporal and Demographic Trends in Glomerular Disease Epidemiology in the Southeastern United States, 1986-2015. Clin J Am Soc Nephrol. 2017;12(4):614–23.
45. Klessens CQ, Woutman TD, Veraar KA, Zandbergen M, Valk EJ, Rotmans JI, et al. An autopsy study suggests that diabetic nephropathy is underdiagnosed. Kidney Int. 2016;90(1):149–56.
46. Lemley KV, Abdullah I, Myers BD, Meyer TW, Blouch K, Smith WE, et al. Evolution of incipient nephropathy in type 2 diabetes mellitus. Kidney Int. 2000;58(3):1228–37.
47. Osterby R, Gall MA, Schmitz A, Nielsen FS, Nyberg G, Parving HH. Glomerular structure and function in proteinuric type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993;36(10):1064–70.
48. Ekinci EI, Jerums G, Skene A, Crammer P, Power D, Cheong KY, et al. Renal structure in normoalbuminuric and albuminuric patients with type 2 diabetes and impaired renal function. Diabetes Care. 2013;36(11):3620–6.
49. Shimizu M, Furuichi K, Toyama T, Kitajima S, Hara A, Kitagawa K, et al. Long-term outcomes of Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy. Diabetes Care. 2013;36(11):3655–62.
50. Nosadini R, Velussi M, Brocco E, Bruseghin M, Abaterusso C, Saller A, et al. Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. Diabetes. 2000;49(3):476–84.
51. Christensen PK, Larsen S, Horn T, Olsen S, Parving HH. Renal function and structure in albuminuric type 2 diabetic patients without retinopathy. Nephrol Dial Transplant. 2001;16(12):2337–47.
52. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, et al. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia. 1996;39(12):1569–76.
53. Pagtalunan ME, Miller PL, Jumping-Eagle S, Nelson RG, Myers BD, Rennke HG, et al. Podocyte loss and progressive glomerular injury in type II diabetes. J Clin Invest. 1997;99(2):342–8.
54. White KE, Bilous RW. Type 2 diabetic patients with nephropathy show structural-functional relationships that are similar to type 1 disease. J Am Soc Nephrol. 2000;11(9):1667–73.
55. Moriya T, Tanaka K, Hosaka T, Hirasawa Y, Fujita Y. Renal structure as an indicator for development of albuminuria in normo- and microalbuminuric type 2 diabetic patients. Diabetes Res Clin Pract. 2008;82(3):298–304.
56. Fufaa GD, Weil EJ, Lemley KV, Knowler WC, Brosius FC 3rd, Yee B, et al. Structural Predictors of Loss of Renal Function in American Indians with Type 2 Diabetes. Clin J Am Soc Nephrol. 2016;11(2):254–61.
57. Lioudaki E, Stylianou KG, Petrakis I, Kokologiannakis G, Passam A, Mikhailidis DP, et al. Increased Urinary Excretion of Podocyte Markers in Normoalbuminuric Patients with Diabetes. Nephron. 2015;131(1):34–42.
58. Nakamura T, Ushiyama C, Suzuki S, Hara M, Shimada N, Ebihara I, et al. Urinary excretion of podocytes in patients with diabetic nephropathy. Nephrol Dial Transplant. 2000;15(9):1379–83.
59. Dalla Vestra M, Masiero A, Roiter AM, Saller A, Crepaldi G, Fioretto P. Is podocyte injury relevant in diabetic nephropathy? Studies in patients with type 2 diabetes. Diabetes. 2003;52(4):1031–5.
60. White KE, Bilous RW. Structural alterations to the podocyte are related to proteinuria in type 2 diabetic patients. Nephrol Dial Transplant. 2004;19(6):1437–40.
61. Meyer TW, Bennett PH, Nelson RG. Podocyte number predicts long-term urinary albumin excretion in Pima Indians with Type II diabetes and microalbuminuria. Diabetologia. 1999;42(11):1341–4.
62. Fiorentino M, Bolignano D, Tesar V, Pisano A, Van Biesen W, D'Arrigo G, et al. Renal Biopsy in 2015--From Epidemiology to Evidence-Based Indications. Am J Nephrol. 2016;43(1):1–19.

63. Gambara V, Mecca G, Remuzzi G, Bertani T. Heterogeneous nature of renal lesions in type II diabetes. J Am Soc Nephrol. 1993;3(8):1458–66.
64. Parving HH, Gall MA, Skott P, Jorgensen HE, Lokkegaard H, Jorgensen F, et al. Prevalence and causes of albuminuria in non-insulin-dependent diabetic patients. Kidney Int. 1992;41(4):758–62.
65. Pham TT, Sim JJ, Kujubu DA, Liu IL, Kumar VA. Prevalence of nondiabetic renal disease in diabetic patients. Am J Nephrol. 2007;27(3):322–8.
66. Haas M, Racusen LC, Bagnasco SM. IgA-dominant postinfectious glomerulonephritis: a report of 13 cases with common ultrastructural features. Hum Pathol. 2008;39(9):1309–16.
67. Nasr SH, Markowitz GS, Stokes MB, Said SM, Valeri AM, D'Agati VD. Acute postinfectious glomerulonephritis in the modern era: experience with 86 adults and review of the literature. Medicine (Baltimore). 2008;87(1):21–32.
68. Gonzalez Suarez ML, Thomas DB, Barisoni L, Fornoni A. Diabetic nephropathy: Is it time yet for routine kidney biopsy? World J Diabetes. 2013;4(6):245–55.
69. Sharma SG, Bomback AS, Radhakrishnan J, Herlitz LC, Stokes MB, Markowitz GS, et al. The modern spectrum of renal biopsy findings in patients with diabetes. Clin J Am Soc Nephrol. 2013;8(10):1718–24.
70. Mazzucco G, Bertani T, Fortunato M, Bernardi M, Leutner M, Boldorini R, et al. Different patterns of renal damage in type 2 diabetes mellitus: a multicentric study on 393 biopsies. Am J Kidney Dis. 2002;39(4):713–20.
71. Oh SW, Kim S, Na KY, Chae DW, Jin DC, Chin HJ. Clinical implications of pathologic diagnosis and classification for diabetic nephropathy. Diabetes Res Clin Pract. 2012;97(3):418–24.
72. Schwartz MM, Lewis EJ, Leonard-Martin T, Lewis JB, Batlle D. Renal pathology patterns in type II diabetes mellitus: relationship with retinopathy. The Collaborative Study Group. Nephrol Dial Transplant. 1998;13(10):2547–52.
73. Zhuo L, Ren W, Li W, Zou G, Lu J. Evaluation of renal biopsies in type 2 diabetic patients with kidney disease: a clinicopathological study of 216 cases. Int Urol Nephrol. 2013;45(1):173–9.
74. Olsen S, Mogensen CE. How often is NIDDM complicated with non-diabetic renal disease? An analysis of renal biopsies and the literature. Diabetologia. 1996;39(12):1638–45.
75. Dai DF, Sasaki K, Lin MY, Smith KD, Nicosia RF, Alpers CE, et al. Interstitial eosinophilic aggregates in diabetic nephropathy: allergy or not? Nephrol Dial Transplant. 2015;30(8):1370–6.
76. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21(4):556–63.
77. An Y, Xu F, Le W, Ge Y, Zhou M, Chen H, et al. Renal histologic changes and the outcome in patients with diabetic nephropathy. Nephrol Dial Transplant. 2015;30(2):257–66.
78. Okada T, Nagao T, Matsumoto H, Nagaoka Y, Wada T, Nakao T. Histological predictors for renal prognosis in diabetic nephropathy in diabetes mellitus type 2 patients with overt proteinuria. Nephrology (Carlton). 2012;17(1):68–75.
79. Li L, Zhang X, Li Z, Zhang R, Guo R, Yin Q, et al. Renal pathological implications in type 2 diabetes mellitus patients with renal involvement. J Diabetes Complications. 2017;31(1):114–21.
80. Hoshino J, Mise K, Ueno T, Imafuku A, Kawada M, Sumida K, et al. A pathological scoring system to predict renal outcome in diabetic nephropathy. Am J Nephrol. 2015;41(4-5):337–44.
81. Mauer SM, Steffes MW, Sutherland DE, Najarian S, Michael AF, Brown DM. Studies of the rate of regression of the glomerular lesions in diabetic rats treated with pancreatic islet transplantation. Diabetes. 1975;24(3):280–5.
82. Pichaiwong W, Hudkins KL, Wietecha T, Nguyen TQ, Tachaudomdach C, Li W, et al. Reversibility of structural and functional damage in a model of advanced diabetic nephropathy. J Am Soc Nephrol. 2013;24(7):1088–102.
83. Fioretto P, Mauer SM, Bilous RW, Goetz FC, Sutherland DE, Steffes MW. Effects of pancreas transplantation on glomerular structure in insulin-dependent diabetic patients with their own kidneys. Lancet. 1993;342(8881):1193–6.

84. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, et al. Recruitment of podocytes from glomerular parietal epithelial cells. J Am Soc Nephrol. 2009;20(2):333–43.
85. Ronconi E, Sagrinati C, Angelotti ML, Lazzeri E, Mazzinghi B, Ballerini L, et al. Regeneration of glomerular podocytes by human renal progenitors. J Am Soc Nephrol. 2009;20(2):322–32.
86. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, et al. Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. J Am Soc Nephrol. 2006;17(9):2443–56.
87. Zhang J, Pippin JW, Krofft RD, Naito S, Liu ZH, Shankland SJ. Podocyte repopulation by renal progenitor cells following glucocorticoids treatment in experimental FSGS. Am J Physiol Renal Physiol. 2013;304(11):F1375–89.
88. Mauer M, Zinman B, Gardiner R, Suissa S, Sinaiko A, Strand T, et al. Renal and retinal effects of enalapril and losartan in type 1 diabetes. N Engl J Med. 2009;361(1):40–51.
89. Weil EJ, Fufaa G, Jones LI, Lovato T, Lemley KV, Hanson RL, et al. Effect of losartan on prevention and progression of early diabetic nephropathy in American Indians with type 2 diabetes. Diabetes. 2013;62(9):3224–31.
90. Brosius FC, 3rd, Alpers CE, Bottinger EP, Breyer MD, Coffman TM, Gurley SB, et al. Mouse models of diabetic nephropathy. J Am Soc Nephrol. 2009;20(12):2503–12.
91. Alpers CE, Hudkins KL. Mouse models of diabetic nephropathy. Curr Opin Nephrol Hypertens. 2011;20(3):278–84.
92. Breyer MD, Bottinger E, Brosius FC, 3rd, Coffman TM, Harris RC, Heilig CW, et al. Mouse models of diabetic nephropathy. J Am Soc Nephrol 2005;16(1):27-45.
93. Bolzan AD, Bianchi MS. Genotoxicity of streptozotocin. Mutat Res. 2002;512(2-3):121–34.
94. Like AA, Appel MC, Williams RM, Rossini AA. Streptozotocin-induced pancreatic insulitis in mice. Morphologic and physiologic studies. Lab Invest. 1978;38(4):470–86.
95. Kitada M, Ogura Y, Koya D. Rodent models of diabetic nephropathy: their utility and limitations. Int J Nephrol Renovasc Dis. 2016;9:279–90.
96. Wang J, Takeuchi T, Tanaka S, Kubo SK, Kayo T, Lu D, et al. A mutation in the insulin 2 gene induces diabetes with severe pancreatic beta-cell dysfunction in the Mody mouse. J Clin Invest. 1999;103(1):27–37.
97. Gurley SB, Mach CL, Stegbauer J, Yang J, Snow KP, Hu A, et al. Influence of genetic background on albuminuria and kidney injury in Ins2(+/C96Y) (Akita) mice. Am J Physiol Renal Physiol. 2010;298(3):F788–95.
98. Haseyama T, Fujita T, Hirasawa F, Tsukada M, Wakui H, Komatsuda A, et al. Complications of IgA nephropathy in a non-insulin-dependent diabetes model, the Akita mouse. Tohoku J Exp Med. 2002;198(4):233–44.
99. Xu J, Huang Y, Li F, Zheng S, Epstein PN. FVB mouse genotype confers susceptibility to OVE26 diabetic albuminuria. Am J Physiol Renal Physiol. 2010;299(3):F487–94.
100. Teiken JM, Audettey JL, Laturnus DI, Zheng S, Epstein PN, Carlson EC. Podocyte loss in aging OVE26 diabetic mice. Anat Rec (Hoboken). 2008;291(1):114–21.
101. Leiter EH, Prochazka M, Coleman DL. The non-obese diabetic (NOD) mouse. Am J Pathol. 1987;128(2):380–3.
102. Atkinson MA, Leiter EH. The NOD mouse model of type 1 diabetes: as good as it gets? Nat Med. 1999;5(6):601–4.
103. Azushima K, Gurley SB, Coffman TM. Modelling diabetic nephropathy in mice. Nat Rev Nephrol. 2018;14(1):48–56.
104. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. Science. 1966;153(3740):1127–8.
105. Koya D, Haneda M, Nakagawa H, Isshiki K, Sato H, Maeda S, et al. Amelioration of accelerated diabetic mesangial expansion by treatment with a PKC beta inhibitor in diabetic db/db mice, a rodent model for type 2 diabetes. FASEB J. 2000;14(3):439–47.
106. Taketomi S, Ikeda H, Ishikawa E, Iwatsuka H. Determination of overall insulin sensitivity in diabetic mice, KK. Horm Metab Res. 1982;14(1):14–8.

107. Matsuo T, Shino A. Induction of diabetic alterations by goldthioglucose-obesity in KK,ICR and C57BL mice. Diabetologia. 1972;8(6):391–7.
108. Qi Z, Fujita H, Jin J, Davis LS, Wang Y, Fogo AB, et al. Characterization of susceptibility of inbred mouse strains to diabetic nephropathy. Diabetes. 2005;54(9):2628–37.
109. Omote K, Gohda T, Murakoshi M, Sasaki Y, Kazuno S, Fujimura T, et al. Role of the TNF pathway in the progression of diabetic nephropathy in KK-A(y) mice. Am J Physiol Renal Physiol. 2014;306(11):F1335–47.
110. Ito T, Tanimoto M, Yamada K, Kaneko S, Matsumoto M, Obayashi K, et al. Glomerular changes in the KK-Ay/Ta mouse: a possible model for human type 2 diabetic nephropathy. Nephrology (Carlton). 2006;11(1):29–35.
111. Shiota M, Printz RL. Diabetes in Zucker diabetic fatty rat. Methods Mol Biol. 2012;933:103–23.
112. Clark JB, Palmer CJ, Shaw WN. The diabetic Zucker fatty rat. Proc Soc Exp Biol Med. 1983;173(1):68–75.
113. Chander PN, Gealekman O, Brodsky SV, Elitok S, Tojo A, Crabtree M, et al. Nephropathy in Zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen. J Am Soc Nephrol. 2004;15(9):2391–403.
114. Ikeda H, Shino A, Matsuo T, Iwatsuka H, Suzuoki Z. A new genetically obese-hyperglycemic rat (Wistar fatty). Diabetes. 1981;30(12):1045–50.
115. Kitada M, Ogura Y, Suzuki T, Sen S, Lee SM, Kanasaki K, et al. A very-low-protein diet ameliorates advanced diabetic nephropathy through autophagy induction by suppression of the mTORC1 pathway in Wistar fatty rats, an animal model of type 2 diabetes and obesity. Diabetologia. 2016;59(6):1307–17.
116. Nagai N, Murao T, Ito Y, Okamoto N, Sasaki M. Enhancing effects of sericin on corneal wound healing in Otsuka Long-Evans Tokushima fatty rats as a model of human type 2 diabetes. Biol Pharm Bull. 2009;32(9):1594–9.
117. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T. Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes. 1992;41(11):1422–8.
118. Portha B, Serradas P, Bailbe D, Suzuki K, Goto Y, Giroix MH. Beta-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. Diabetes. 1991;40(4):486–91.
119. Ostenson CG, Khan A, Abdel-Halim SM, Guenifi A, Suzuki K, Goto Y, et al. Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. Diabetologia. 1993;36(1):3–8.
120. Yagihashi S, Goto Y, Kakizaki M, Kaseda N. Thickening of glomerular basement membrane in spontaneously diabetic rats. Diabetologia. 1978;15(4):309–12.
121. Feng B, Yan XF, Xue JL, Xu L, Wang H. The protective effects of alpha-lipoic acid on kidneys in type 2 diabetic Goto-Kakisaki rats via reducing oxidative stress. Int J Mol Sci. 2013;14(4):6746–56.
122. Nobrega MA, Fleming S, Roman RJ, Shiozawa M, Schlick N, Lazar J, et al. Initial characterization of a rat model of diabetic nephropathy. Diabetes. 2004;53(3):735–42.
123. Kojima N, Slaughter TN, Paige A, Kato S, Roman RJ, Williams JM. Comparison of the development diabetic induced renal disease in strains of Goto-Kakizaki rats. J Diabetes Metab. 2013;Suppl 9(5):S9–005.
124. Zhao HJ, Wang S, Cheng H, Zhang MZ, Takahashi T, Fogo AB, et al. Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. J Am Soc Nephrol. 2006;17(10):2664–9.
125. Mohan S, Reddick RL, Musi N, Horn DA, Yan B, Prihoda TJ, et al. Diabetic eNOS knockout mice develop distinct macro- and microvascular complications. Lab Invest. 2008;88(5):515–28.
126. Thibodeau JF, Holterman CE, Burger D, Read NC, Reudelhuber TL, Kennedy CR. A novel mouse model of advanced diabetic kidney disease. PLoS One. 2014;9(12):e113459.

127. Clee SM, Nadler ST, Attie AD. Genetic and genomic studies of the BTBR ob/ob mouse model of type 2 diabetes. Am J Ther. 2005;12(6):491–8.
128. Hudkins KL, Pichaiwong W, Wietecha T, Kowalewska J, Banas MC, Spencer MW, et al. BTBR Ob/Ob mutant mice model progressive diabetic nephropathy. J Am Soc Nephrol. 2010;21(9):1533–42.
129. Gembardt F, Bartaun C, Jarzebska N, Mayoux E, Todorov VT, Hohenstein B, et al. The SGLT2 inhibitor empagliflozin ameliorates early features of diabetic nephropathy in BTBR ob/ob type 2 diabetic mice with and without hypertension. Am J Physiol Renal Physiol. 2014;307(3):F317–25.
130. Gurley SB, Clare SE, Snow KP, Hu A, Meyer TW, Coffman TM. Impact of genetic background on nephropathy in diabetic mice. Am J Physiol Renal Physiol. 2006;290(1):F214–22.
131. Chang JH, Paik SY, Mao L, Eisner W, Flannery PJ, Wang L, et al. Diabetic kidney disease in FVB/NJ Akita mice: temporal pattern of kidney injury and urinary nephrin excretion. PLoS One. 2012;7(4):e33942.
132. Zheng S, Noonan WT, Metreveli NS, Coventry S, Kralik PM, Carlson EC, et al. Development of late-stage diabetic nephropathy in OVE26 diabetic mice. Diabetes. 2004;53(12):3248–57.
133. Yuzawa Y, Niki I, Kosugi T, Maruyama S, Yoshida F, Takeda M, et al. Overexpression of calmodulin in pancreatic beta cells induces diabetic nephropathy. J Am Soc Nephrol. 2008;19(9):1701–11.
134. Chua S Jr, Li Y, Liu SM, Liu R, Chan KT, Martino J, et al. A susceptibility gene for kidney disease in an obese mouse model of type II diabetes maps to chromosome 8. Kidney Int. 2010;78(5):453–62.
135. Wang Z, Jiang T, Li J, Proctor G, McManaman JL, Lucia S, et al. Regulation of renal lipid metabolism, lipid accumulation, and glomerulosclerosis in FVBdb/db mice with type 2 diabetes. Diabetes. 2005;54(8):2328–35.
136. Sharma K, McCue P, Dunn SR. Diabetic kidney disease in the db/db mouse. Am J Physiol Renal Physiol. 2003;284(6):F1138–44.
137. McCarthy KJ, Routh RE, Shaw W, Walsh K, Welbourne TC, Johnson JH. Troglitazone halts diabetic glomerulosclerosis by blockade of mesangial expansion. Kidney Int. 2000;58(6):2341–50.
138. Katsuda Y, Ohta T, Miyajima K, Kemmochi Y, Sasase T, Tong B, et al. Diabetic complications in obese type 2 diabetic rat models. Exp Anim. 2014;63(2):121–32.
139. Azushima K, Gurley SB, Coffman TM. Modelling diabetic nephropathy in mice. Nat Rev Nephrol. 2018;14(1):48–56.