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Original Article

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Structural alterations to the podocyte are related to proteinuria in type 2 diabetic patients

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Abstract

Background. The podocyte is believed to play a key role in maintaining the integrity of the glomerular filtration barrier, and damage or loss has been linked to the development of albuminuria.

Methods. Renal biopsies from 16 type 2 diabetic patients with nephropathy and 28 non-diabetic controls were analysed using light and electron microscopy.

Results. Podocyte number per glomerulus was significantly lower in the type 2 patients compared with controls [mean (95% confidence interval) 464 (382–546) *vs* 589 (543–635), $P = 0.004$]. Mean glomerular volume was significantly increased in diabetic patients compared with controls [5.5 (4.9–6.1) *vs* 3.1 (2.7–3.5) $\times 10^6\,\mu m^3$, $P < 0.001$], thus the diabetic patients demonstrated an even greater proportional reduction in podocyte density per glomerulus [88 (68–108) *vs* 201 (182–220)/$10^6\,\mu m^3$, $P < 0.001$]. Podocyte foot process width on both the filtration surface (FPWgbm) and mesangial surface (FPWmes) was significantly increased compared with controls [796 (708–884) *vs* 556 (460–908) nm, $P = 0.001$; 1108 (821–1394) *vs* 760 (555–1078) nm, $P = 0.029$, respectively]. There was a significant negative correlation between proteinuria and both podocyte number and podocyte density per glomerulus ($r = -0.63$, $P = 0.009$; $r = -0.58$, $P = 0.018$, respectively). There was a significant positive correlation between proteinuria and both FPWgbm and FPWmes ($r = 0.64$, $P = 0.008$, for both).

Conclusion. Podocyte loss occurs in type 2 diabetic nephropathy and is related to increasing proteinuria. Whether the accompanying glomerular enlargement and widening of foot processes are a cause of podocyte loss is uncertain. Longitudinal studies are required to determine the sequence of events leading to podocyte loss in diabetic nephropathy.

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Introduction

Diabetic nephropathy is characterized by a decline in glomerular filtration rate (GFR), and increasing proteinuria and systemic blood pressure. The relationships between the structural lesions of glomerulopathy and renal function are well documented [1–4]. However, although the decline in GFR can be explained in part by the loss of total available filtration surface area per glomerulus [5], the structural mechanisms behind proteinuria are less clearly understood.

Recent studies in both type 1 and type 2 diabetes [6–10] and other nephropathies [11] have proposed that a reduction in the number of podocytes may lead to the development of proteinuria. These workers have deduced that, as a result of the remaining cells having to cover a greater surface area, there is disruption of cell integrity and increased leakiness of the filtration barrier. However, despite an overall reduction in podocyte density, we could not confirm reduced podocyte numbers in normotensive type 1 patients with early nephropathy [12].

Most of the previously published studies in type 2 diabetes have been performed in Pima Indians [6–8], with only one study in Caucasian patients [10]. The present study adds to the limited data available on podocyte number in human type 2 diabetes by reporting results from a group of 16 patients with nephropathy.

Subjects and methods

The type 2 diabetic patients were part of a study assessing the effects of perindopril on renal structure, and eligibility criteria have been published previously [13]. All studies were performed in accordance with the guidelines proposed in the

Declaration of Helsinki. Approval was obtained from local independent review bodies and informed signed consent was obtained from each patient.

Only 16 patients who had sufficient biopsy material for morphometric analysis by both light and electron microscopy were included in the present study. There were no clinical differences between these patients and the whole cohort of 22 patients (Table 1). The total biopsy series examined by light microscopy has been described in detail elsewhere [13]. Type 2 patients with non-diabetic glomerulopathy were excluded. Fourteen out of the 16 patients were receiving anti-hypertensive treatment and nine were receiving insulin therapy. Data presented are from biopsies performed at the start of the study, prior to randomization.

Biopsy material for both light and electron microscopy was available from 10 non-diabetic kidney donors at the time of transplantation (mean age 38 years, five male). As these control subjects were significantly younger than our type 2 patients, we also estimated podocyte number and density in an older group of 18 subjects (mean age 67 years, 10 male) who underwent nephrectomy for tumours.

Clinical measurements

The clinical methods have been reported previously [13]. Briefly, blood pressure was measured as the average of three readings taken 1 min apart using a calibrated mercury sphygmomanometer. All urine passed during a 24-h period was collected for creatinine clearance and urine protein excretion. Creatinine clearance was assessed by a colorimetric method using picric acid. Urine protein concentration was determined by a colorimetric method using pyrogallol red.

Table 1. Clinical characteristics of 16 type 2 diabetic patients and the original cohort of 22

Number (M/F)	16 (13/3)	22 (18/4)
Age (years)	48 (42–53)	47 (43–51)
Creatinine clearance (ml/min/1.73 m^2)	124 (99–148)	123 (105–141)
Proteinuria (mg/24 h)[a]	570 (73–3877)	600 (70–4200)
Systolic BP (mmHg)	146 (136–157)	144 (136–152)
Diastolic BP (mmHg)	85 (80–89)	86 (81–90)

Data are mean (95%CI) or [a]median (range).

Laboratory methods

Biopsy material was fixed in glutaraldehyde, embedded in epoxy resin and sectioned for light and electron microscopy as previously described [4]. Light microscopy was used to estimate mean glomerular volume using the Cavalieri method on five glomeruli per biopsy [14], and podocyte number by the disector/fractionator method [15] on three glomeruli per biopsy.

Electron microscopy was used to estimate capillary length, surface areas and podocyte foot process width (FPW). Estimations were performed using standard stereological techniques as previously described [4,16]. The surface areas measured were the mesangio-urinary surface area and peripheral GBM or filtration surface area. The sum of these two surface areas was also calculated in order to estimate the total surface area of GBM underlying the podocytes.

Statistical analyses

Values for proteinuria were not normally distributed and were logarithmically transformed. Analysis was carried out using SPSS 11.0. Comparisons between groups were performed using Student's *t*-test. Relationships between parameters were analysed using Pearson's correlation coefficient. Stepwise linear regression was performed using proteinuria as the outcome variable. A two-tailed P-value < 0.05 was considered statistically significant.

Results

The clinical characteristics of the 16 patients included in this analysis are shown in Table 1. The results of the structural analyses are shown in Table 2. Podocyte number per glomerulus was significantly lower

Table 2. Structural parameters in type 2 diabetic patients compared with non-diabetic controls

	Type 2 $n = 16$	Non-diabetic $n = 28$ LM, $n = 10$ EM	P
Podo/glom	464 (382–546)	589 (543–634)	0.004
NvPodo/10^6 μm^3	88 (68–108)	201 (182–220)	< 0.001
MGV $\times$ 10^6 μm^3	5.5 (4.9–6.1)	3.1 (2.7–3.5)	< 0.001
GBMsurf/Glom mm^2[a]	0.82 (0.67–0.96)	0.55 (0.46–0.63)	< 0.01
CapL/Glom mm[a]	44 (39–50)	26 (21–30)	< 0.001
FPWgbm nm[a]	796 (708–884)	546 (480–908)	< 0.01
FPWmes nm[a]	1108 (821–1394)	769 (555–1078)	< 0.05

Data are mean (95% confidence interval).
[a]Parameters measured by electron microscopy.
LM = light microscopy; EM = electron microscopy; Podo/Glom = podocyte number per glomerulus; NvPodo = podocyte density per glomerulus; MGV = mean glomerular volume; GBMsurf/Glom = glomerular basement membrane surface area underlying podocytes (filtration surface area + mesangio-urinary surface area per glomerulus); CapL/Glom = capillary length per glomerulus; FPWgbm = foot process width on peripheral glomerular basement membrane; FPWmes = foot process width on mesangio-urinary surface.

in the diabetic patients compared with controls (20% reduction). Mean glomerular volume, GBM surface area underlying the podocytes, and capillary length were all significantly greater in the type 2 patients compared with controls. Thus the diabetic patients demonstrated a proportionally greater reduction (46%) in podocyte density per glomerulus. FPW on both the peripheral GBM (FPWgbm) and mesangio-urinary surface (FPWmes) was significantly greater in the diabetic patients compared with controls.

There was a significant negative correlation between proteinuria and both podocyte number and podocyte density per glomerulus ($r = -0.63$, $P = 0.009$; $r = -0.58$, $P = 0.018$, respectively) (Figure 1). There was a significant positive correlation between both FPWgbm and FPWmes and proteinuria ($r = 0.64$, $P = 0.008$, for both).

Stepwise linear regression showed that FPW was the strongest predictor of proteinuria ($F = 11.3$, multiple $r = 0.67$, $P = 0.005$).

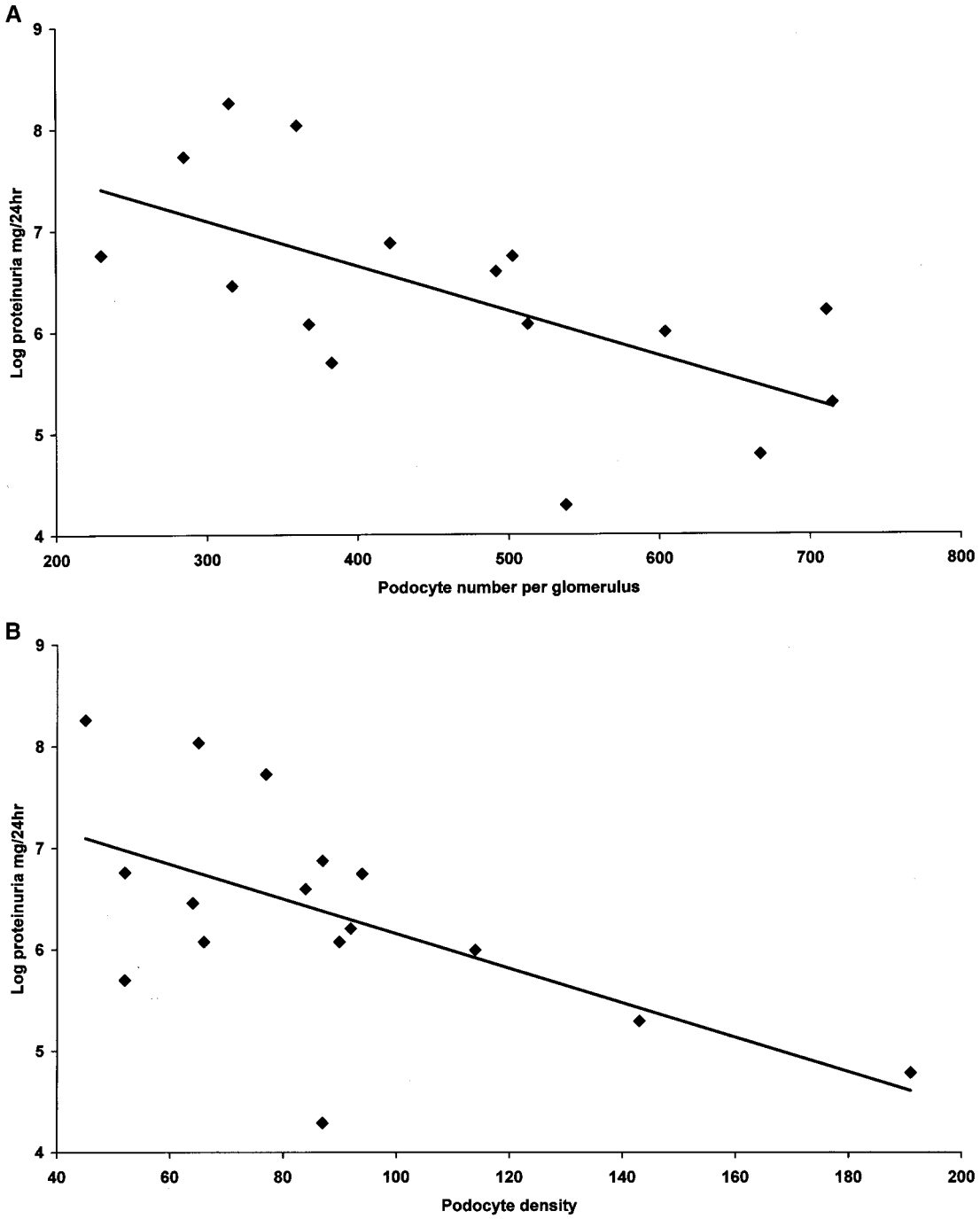

Fig. 1. Plot of total proteinuria (y-axis, logarithmic scale) against (**A**) podocyte number per glomerulus (x-axis), $r = -0.63$, $P = 0.009$; and (**B**) podocyte density per glomerulus/10^6 μm^3 (x-axis), $r = -0.58$, $P = 0.018$, in 16 type 2 diabetic patients.

Discussion

Structural alterations to the components of the filtration barrier are likely to contribute to increased albumin leakage, but it is difficult to determine the exact sequence of events that leads to the breakdown of its integrity. Any disruption to the filtration barrier that affects its size or charge selectivity would result in increased passage of molecules such as albumin. If there were a loss of podocytes, this would lead to an expansion of foot processes from the remaining podocytes. This in turn would tend to widen the filtration slits and perhaps disrupt the slit membrane. These mechanical events might then adversely affect permselectivity, leading to proteinuria.

Our data support this hypothesis and are consistent with data from the Pima Indians and from Italy [6,7,10]. We found a reduction in podocyte number per glomerulus, associated with an increase in FPW and a negative correlation with proteinuria.

However, it is possible that podocyte loss is not the cause of foot process widening as outlined above, but that it is the increases in glomerular volume, capillary length and GBM surface area seen in our diabetic patients which result in widening of the foot processes as each podocyte has to cover a greater surface area. This then leads to a loss of cell integrity, leakiness of the filtration barrier and ultimately to podocyte loss. Several processes may lead to mechanical stretch: raised intracapillary pressure perhaps secondary to systemic hypertension; compensatory capillary elongation in response to glomerular loss secondary to diabetes; or disruption of the GBM–mesangial ultrastructural connections due to matrix accumulation.

Our type 2 patients had high blood pressure, large glomeruli and significant mesangial matrix expansion, all of which may have contributed to podocyte loss. These parameters were significantly more abnormal than in our previously described type 1 cohort, who did not demonstrate a reduction in podocyte number per glomerulus [12]. Thus, podocyte loss may be more of a late feature of clinical nephropathy, occurring only when other factors such as hypertension are present.

In conclusion, although it is evident that podocyte loss occurs in type 2 diabetes and is related to proteinuria, cause and effect cannot be determined. Longitudinal studies are required in order to determine the precise sequence of events and the role of podocyte loss in progression of diabetic nephropathy.

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Conflict of interest statement. None declared.

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