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Serial renal biopsies in normo- and microalbuminuric patients with type 2 diabetes demonstrate that loss of renal function is associated with a reduction in glomerular filtration surface secondary to mesangial expansion

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ABSTRACT

Aims: The aim of the present study was to explore the relationship between changes in renal structure in patients with type 2 diabetes at an early stage of diabetic nephropathy using serial renal biopsies, and change in renal function.
Methods: The study population comprised 10 patients with type 2 diabetes with normo- or microalbuminuria at baseline. Light and electron microscopy-based morphometric analyses were performed to quantitatively evaluate glomerular and interstitial structural changes. Urinary albumin excretion (UAE) and glomerular filtration rate (GFR) were measured annually. A second renal biopsy was performed after a mean of 6.1 ± 2.4 years of follow-up.
Results: UAE, GFR, blood pressure and severity of diabetic retinopathy (DR) did not change between the baseline and follow-up. The annual decrease in the surface density of the peripheral glomerular basement membrane (GBM) (Sv[PGBM/glom]) was correlated with the rate of loss of GFR during the six-year follow-up period. The annual change in the Sv(PGBM/glom) was negatively correlated with the change in mesangial volume fraction.
Conclusions: Decreases in the GFR in patients with type 2 diabetes with normo- or microalbuminuria at baseline were associated with a decreased glomerular filtration surface, as a result of mesangial expansion during a mean six years of observation. These findings confirm ongoing pathological progression of glomerulopathy despite no significant change in albuminuria or retinopathy status.

1. Introduction

In types 1 and 2 diabetes, the clinical manifestations of diabetic nephropathy include an increase in albuminuria and a decline in renal function. Several longitudinal studies have shown that histological parameters can be used to predict a decline in renal function or an increase in albuminuria in patients with both type 1 and 2 diabetes.[1,2] Moreover, a recent report showed that glomerular basement membrane (GBM) thickening was a risk factor for the subsequent development of macroalbuminuria or end-stage kidney disease in normoalbuminuric patients with type 1 diabetes.[1] In Japanese patients with type 2 diabetes, GBM thickening and mesangial expansion predicted an increase in albuminuria after six years of follow-up[2].

GBM thickening and mesangial expansion have been shown to predict a decline in GFR in micro- and macroalbuminuric patients with type 2 diabetes.[3] More recently, a research renal biopsy study[4] of Native American patients with type 2 diabetes and mainly normo- and microalbuminuria revealed that changes in the glomerular structure, including mesangial expansion, predicted a reduction in renal function. Several recent reports in Japanese[5,6] and Chinese[7] patients with type 2 diabetes have shown correlations between glomerular structure and renal function although most of the patients in these studies were macroalbuminuric.

The above-mentioned studies mostly used a single baseline renal biopsy in order to derive structural predictors of renal function changes during follow-up. These data should therefore be interpreted with caution; for example, in one report, arteriolar hyalinosis, was associated with an increase in albuminuria and a decline in renal function.[8] However, whether or not arteriolar hyalinosis was actually the cause of these clinical changes remains unknown. Urinary albumin excretion (UAE) increased in parallel with mesangial expansion in a study using

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serial biopsies in patients with type 1 diabetes,[9] but GFR was not reported. A Japanese study[10] from 1975 demonstrated a relationship between glycemic control and changes in the renal histology using a serial renal biopsy. However, the study[10] did not define the type of diabetes or provide details about renal function at the follow-up examination. Thus, to our knowledge, although the course of renal structural-functional relationships in type 1 diabetes has been relatively well described,[11] there are few data in patients with type 2, particularly in those at an early stage of nephropathy.

The effects of renin-angiotensin-aldosterone system (RAAS) blockade on retinal and renal changes have been described[12]; however, that report did not describe the detailed relationships between changes in the renal histology and function over a long observational period. Thus, the histological findings associated with a decline in renal function or an increase in albuminuria in patients with type 2 diabetes remains relatively unknown.

We therefore conducted the present analysis to evaluate the course of renal structural-functional relationships using serial renal biopsies in Japanese patients with type 2 diabetes, in order to identify any histological findings that might predict a decline in renal function in those with normo- or microalbuminuria at baseline.

2. Subjects, materials and methods

2.1. Participants - type 2 diabetic patients

Normotensive patients with type 2 diabetes, aged 20–60 years old without macroalbuminuria, hematuria or a serum creatinine concentration of >2.0 mg/dl (176.8 μmol/l) and without any evidence suggestive of atherosclerotic disease were recruited at the outpatient clinic of Kitasato University Hospital. The following patients were excluded from this initial recruitment: patients who were receiving antihypertensive drugs; patients with a history of any malignant, cerebrovascular, or cardiovascular disease; and patients with recurrent infection.

All of the patients were fully informed about the trial, and 30 Japanese patients with type 2 diabetes who gave their consent to participate in the present study underwent percutaneous research renal biopsies. They showed no evidence of non-diabetic renal glomerular or tubular/interstitial changes. Some of the patients who were included in the present study had been included in some of our previous studies[2,8,13,14]; we excluded patients who had been included in previous studies if they underwent biopsies for clinical or diagnostic indications. Of these original 30 patients, 10 participated in the present study and underwent repeat biopsy.

The study population comprised 3 women and 7 men (age, 48 ± 8 years) in whom the known duration of diabetes was 12 ± 8 years.

There were no significant differences in the baseline clinical parameters or the morphometric data between the 10 patients who underwent a serial biopsy and the remaining 20 who underwent a single biopsy. Normoalbuminuria (<20 μg/min) and microalbuminuria (20–200 μg/min) were defined based on the UAE measured in 4-h timed urine samples that were obtained after an injection of iohexol for estimation of GFR. Clinical examination was performed along with a renal biopsy, and the patients were followed up annually thereafter for a mean of 6.1 ± 2.4 years.

The protocol for this study was approved by the Research Ethics Committee of Kitasato University School of Medicine and all patients gave informed consent.

2.2. Normal control subjects

The renal biopsy reference values of the normal controls were obtained from 9 living renal transplant donors (male, $n = 2$; female, $n = 7$; age, 51 ± 8 years; body mass index, 23.0 ± 2.7 kg/m^2) at 1 h after transplantation, as previously described.[13,15] All 9 subjects showed normal 75-g oral glucose tolerance test results, negative dipstick proteinuria and a normal blood pressure (BP; 110 ± 6/69 ± 5 mm Hg).

2.3. Laboratory and clinical measurements

HbA_{1c} was measured by high-performance liquid chromatography (HPLC). The value for HbA_{1c} (%) was estimated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated using the formula: HbA_{1c} (%) = HbA_{1c} (the Japan Diabetes Society [JDS]) (%) + 0.4%. This conversion complied with the relational expression of HbA_{1c} (JDS) (%)[16] measured using the previous Japanese standard substance and measurement methods, and HbA_{1c} (NGSP) followed by conversion to HbA_{1c} (the International Federation of Clinical Chemistry). The GFR was measured using the plasma clearance of unlabeled iohexol.[13,17] Urinary albumin was measured using a turbidimetric immunoassay on a timed 4-h sample collected at the time of the GFR measurement. The BP was measured during the initial hospitalization and at all outpatient visits. Hypertension was defined as a systolic BP (SBP) of ≥130 mm Hg, a diastolic BP (DBP) of ≥85 mm Hg and/or treatment with antihypertensive drugs. During the follow-up period, patients who developed hypertension were prescribed antihypertensive agents in order to achieve optimal BP control (<130/80 mm Hg).

2.4. The morphometric analysis of renal biopsy specimens

For the light microscopic (LM) morphometric analyses, renal biopsy specimens from both diabetic patients and controls were fixed in 10% buffered formalin and subjected to periodic acid-Schiff staining. The mean glomerular volume (MGV) on the LM sections was determined at an approximate magnification of ×150 using the point counting method of Weibel and Gomez.[18] The percent global glomerular sclerosis (%GS) was measured as described previously.[19] At least 15 glomerular profiles per patient (24 ± 10, mean ± standard deviation [SD]) were measured to determine the MGV and %GS values. The interstitial volume fraction (Vv[Int/cortex]) was determined on the LM sections at an approximate magnification of ×300 by point-counting images projected onto a white surface with a projection microscope.[20] The index of arteriolar hyalinosis (IAH) score was obtained by estimating the fraction of each arteriolar wall that was replaced by hyaline in one complete LM section.[8,21]

For the electron microscopic morphometric analyses, kidney tissue was cut into cubes (approximately 1 mm^3), fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and postfixed in osmium tetroxide. These specimens were dehydrated in a graded series of ethanol washes and embedded in Quetol 812 (Nissin EM, Inc., Tokyo, Japan). All specimens were cut into thick and 80–90 nm ultrathin sections and studied using a JEOL CX 100 transmission electron microscope (JEOL, Tokyo, Japan) in the Kitasato Bio-Imaging Center. Routine stereologic techniques, which have been previously described,[15,22–24] were used to measure the glomerular basement membrane (GBM) width, mesangial fractional volume (Vv[Mes/glom]), and surface density of the peripheral GBM (Sv[PGBM/glom]). In brief, the GBM width was measured using the orthogonal intercept method,[24] the Vv(Mes/glom) was measured by point counting, and the Sv(PGBM/glom) was measured using the line-intercept method.[15,22,23]

2.5. Statistical analyses

The data are presented as the mean ± standard deviation (SD), except for the UAE and %GS, which is presented as the median (range). The UAE values were logarithmically transformed before the analysis since they were not normally distributed. Clinical and morphological data between the baseline and follow-up were compared using paired *t*-tests or non-parametric tests for data that were not normally distributed. The relationships among the annual changes in the GFR, UAE and the morphological data during the observational period were evaluated using linear and nonlinear regression analyses. *P* values of <0.05 were considered to indicate statistical significance.

Table 1
Demographic data and clinical characteristics of each patient at the renal biopsy and follow-up.

Case No.	Age at the biopsy (years)	Gender	Duration of diabetes at the biopsy	HbA_{1c} (NGSP%/ IFCC mmol/mol)		SBP (mm Hg)		DBP (mm Hg)	
				Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	56	M	20	7.9/62.8	7.4/57.4	142	132	80	71
2	47	M	3	5.8/39.9	7.0/48.6	142	134	86	81
3	50	F	10	8.3/67.2	7.7/60.6	99	112	60	70
4	58	M	18	5.7/38.8	6.4/46.4	121	132	73	82
5	35	M	3	5.3/34.4	5.7/38.8	122	129	78	82
6	46	F	12	8.3/67.2	12.1/108.7	94	100	58	56
7	49	F	15	8.0/63.9	6.7/49.7	116	140	69	78
8	58	M	28	8.2/66.1	7.5/58.5	128	134	73	64
9	34	M	10	8.6/70.5	7.1/54.1	112	107	74	73
10	49	M	5	7.5/58.5	8.6/70.5	130	125	90	79
Mean ± SD	48 ± 8		12 ± 8	7.3 ± 1.2/56.2 ± 13.6	7.6 ± 1.8/59.3 ± 19.3	120.6 ± 16.1	124.5 ± 13.4	74.1 ± 10.0	73.6 ± 8.6

M, male; F, female; HbA_{1c}, hemoglobin A_{1c}; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation.

3. Results

3.1. Clinical characteristics and laboratory findings (Tables 1 and 2)

The baseline and follow-up clinical characteristics of each patient are shown in Table 1. The mean age was 48 years, and the mean known duration of diabetes was 12 years. There were no significant differences between the baseline and follow-up HbA_{1c}, SBP or DBP values of the study population. None of the patients required antihypertensive agents at the initial evaluation. However, two normoalbuminuric patients and one microalbuminuric patient were prescribed RAAS blockers during the observation period.

The renal function and severity of diabetic retinopathy at the baseline and follow-up examinations are shown in Table 2. The median UAE was 6.6 μg/min; 8 patients were normoalbuminuric, and 2 were microalbuminuric at baseline; 6 patients were normoalbuminuric, and 4 were microalbuminuric at follow-up. Two patients with microalbuminuria did not return to normoalbuminuria during the observation period, despite the prescription of RAAS blockers. Although there was no significant difference between the baseline and follow-up GFR values, GFR decreased from baseline to follow-up in eight patients. At baseline, four patients had mild diabetic retinopathy, and two had moderate diabetic retinopathy with post-photocoagulation. The severity of diabetic retinopathy did not change at the final observation.

3.2. Morphometric analyses comparing patients to controls and between baseline and follow-up (Tables 3, 4)

The renal morphometry findings of the 10 diabetic patients, which were obtained by light microscopy (Table 3) and electron microscopy (Table 4), were compatible with early diabetic nephropathy. In comparison to the control subjects (diabetes vs. controls, respectively), the baseline mean MGV (2.72 ± 0.70 vs. 1.40 ± 0.50; $p = 0.0006$), Vv (Int/cortex) (0.20 ± 0.04 vs. 0.10 ± 0.01; $p < 0.0001$), IAH score (1.67 ± 0.73 vs. 1.01 ± 0.04; $p = 0.034$) and GBM width (702 ± 123 vs. 296 ± 43; $p < 0.001$) were increased, while the %GS (0.0 [0.0–11.7] vs. 6.1 [0.0–23.9]; $p = 0.118$), Vv(Mes/glom) (0.22 ± 0.06 vs. 0.19 ± 0.03; $p = 0.255$) and Sv(PGBM/glom) (0.12 ± 0.03 vs. 0.12 ± 0.02; $p = 1.00$) were not statistically different. The MGV, %GS, IAH score, GBM width, Vv(Mes/glom) and Sv(PGBM/glom) did not change significantly during follow-up ($p = 0.978$, 0.285, 0.852, 0.227, 0.428 and 0.558, respectively). However, the Vv(Int/cortex) value did increase significantly during the follow-up period ($p = 0.012$).

3.3. Changes in renal structural-functional relationships

The annual rate of Sv(PGBM/glom) decline was significantly and negatively correlated with the annual increase in Vv(Mes/glom) ($r = 0.68$, $p = 0.030$) (Fig. 1). None of the other morphometric parameters were significantly correlated with each other. No morphometric parameters were correlated with the annual changes in UAE. However, the annual rate of GFR decline was significantly and positively correlated with the annual change in the Sv(PGBM/glom) ($r = 0.79$, $p = 0.006$) (Fig. 2).

4. Discussion

In the present study we have shown that, over a six year period, patients with type 2 diabetes and either normo- or microalbuminuria, demonstrate a reduction in Sv(PGBM/glom) that correlated with an increase in the Vv(Mes/glom). Moreover, the loss of filtration surface was

Table 2
Urinary albumin, GFR and DR in each patient at the renal biopsy and follow-up.

Case No.	UAE (μg/min)		GFR (ml/min/1.73 m^2)		DR	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	5.9	2.9	114.4	91.7	None	None
2	7.2	12.6	92.5	74.5	Moderate, non-proliferative (p)	Moderate, non-proliferative (p)
3	9.7	3.9	137.1	107.5	None	None
4	5.4	46.4	126.4	96.0	Mild	Mild
5	0.6	5.2	140.3	130.1	Mild	Mild
6	1.9	2.4	86.2	111.7	None	None
7	4.7	6.2	100.9	118.8	Moderate, non-proliferative (p)	Moderate, non-proliferative (p)
8	29.0	37.3	126.0	105.2	Mild	Mild
9	8.9	28.1	146.7	135.7	Mild	Mild
10	180.2	151.0	151.0	116.3	None	None
Median (range), mean ± SD	6.6 (0.6–180.2)	9.4 (2.4–151.0)	122.2 ± 22.9	108.7 ± 18.2	–	–

UAE, urinary albumin excretion; GFR, glomerular filtration rate; DR, diabetic retinopathy; P, photocoagulation; SD, standard deviation.

Table 3
Results of light microscopic morphometric analyses in each patient at the renal biopsy and follow-up.

Case No.	MGV ($\times 10^6$ μm^3)		Vv(Int/cortex)		%GS		IAH score	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	2.17	1.84	0.112	0.374	0.0	48.0	1.21	1.30
2	2.29	2.77	0.212	0.221	0.0	16.0	3.43	3.47
3	2.26	0.76	0.270	0.312	9.6	34.0	1.22	1.50
4	ND	1.79	0.174	0.323	0.0	0.0	1.00	1.00
5	3.72	3.28	0.216	0.362	0.0	0.0	1.47	1.09
6	ND	ND	0.180	0.309	0.0	0.0	1.75	1.10
7	2.28	2.87	0.230	0.204	0.0	0.0	1.31	1.00
8	3.51	4.56	0.230	0.266	0.0	0.0	1.25	1.50
9	2.09	2.55	0.210	0.210	0.0	0.0	2.36	1.00
10	3.45	4.19	0.203	0.353	11.7	0.0	1.64	3.00
Mean ± SD	2.72 ± 0.70	2.74 ± 1.19	0.20 ± 0.04	0.29 ± 0.06	0.0 (0.0–11.7)	0.0 (0.0–48.0)	1.67 ± 0.73	1.60 ± 0.89

MGV, mean glomerular volume; Vv(Int/cortex), volume fraction of cortical interstitium; %GS, percent global glomerular sclerosis; IAH, index of arteriolar hyalinosis; SD, standard deviation.

also correlated with a reduction in GFR. To our knowledge, this finding has not been previously reported in patients with type 2 diabetes and early nephropathy using serial renal biopsies. The correlations between structure and GFR decline confirm that significant pathological progression takes place before demonstrable changes in UAE.

In patients with type 1 diabetes, several reports have shown the course of the renal structural-functional relationships using a serial renal biopsy.[9,25,26] In 11 normo- and microalbuminuric patients with type 1 diabetes, the UAE was found to increase in parallel with mesangial expansion over an interval of 5.6 ± 1.6 years.[9] However, that study did not describe the relationships between the renal histological parameters and the decline in renal function over the long term. Another report on 18 microalbuminuric patients with type 1 diabetes showed that the UAE was correlated with the mesangial matrix/glomerular volume fraction in the follow-up biopsy, but changes in renal function were not described during 2–3 years of follow-up.[25] A longer observational study showed that only the mean HbA_{1c} value affected GFR at the end of an eight-year study, whilst no histological parameters were significantly correlated with changes in renal function.[26] Change in renal interstitial fibrosis was reported as a risk factor for the observed reduction in renal function during 4 years of follow-up[27]; however, this study only examined patients with macroalbuminuria (type 1 diabetes, $n = 29$; type 2 diabetes, $n = 19$). Thus, the current study is the first to reveal significant relationships between changes in renal histology and renal function in normo- and microalbuminuric patients with type 2 diabetes over a six-year follow-up period.

Although a cross-sectional study showed that GFR was associated with glomerular filtration surface area in patients with types 1 and 2 diabetes,[13,28,29] to our knowledge, no previous study has shown a correlation between renal function decline and a decrease in the glomerular surface area in type 2 diabetes before increases in UAE.

However, in contrast to our findings with the Sv(PGBM/glom), the Vv(Mes/glom) was not correlated with the GFR at the time of the renal biopsy, nor did it predict longstanding GFR decline. A previous cross-sectional study showed that mesangial expansion was correlated with a decrease in renal function and increased urinary protein excretion in type 2 diabetes patients with macroalbuminuria.[30] Although the Vv(Mes/glom) values were slightly increased in our patients with normo- and microalbuminuria, mesangial expansion occurs strikingly in the overt proteinuric stage.[15] The results of the present study showed that the changes in GFR and Vv(Mes/glom) value were not associated in patients with normo- or microalbuminuric diabetic nephropathy. Thus, at this early stage of diabetic nephropathy in type 2 diabetes, the change in the Sv(PGBM/glom) may be a more predictive indicator of a change in renal function than mesangial expansion alone.

The relationship between mesangial expansion and the decrease in the filtration surface is very important. Over the long term, the mesangial area is seen to expand with increasing diabetes duration in patients with both type 1 and type 2 diabetes.[9,10] It was shown that the total mesangial area was negatively correlated with the glomerular surface area in a cross-sectional study of 45 normo-, micro- and macroalbuminuric patients with type 1 diabetes.[22] However, no reports have shown that the glomerular surface area decreased in parallel with mesangial expansion in the early stages of diabetic nephropathy in either type 1 or type 2 diabetic patients.

None of the 10 patients showed any significant change in their retinopathy status during follow-up. A previous report showed that the grade of diabetic retinopathy was correlated with changes in renal histology of Japanese patients with diabetes.[10] The lack of an association in the present study might have been due to the very early disease stage together with the small number of patients. Although previously it has been shown that the presence of diabetic retinopathy and

Table 4
Findings from electron microscopic morphometric analyses in each patient at the renal biopsy and follow-up.

Case No.	GBM width (nm)		Vv(Mes/glom)		Sv(PGBM/glom)	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
1	645	645	0.20	0.17	0.11	0.14
2	680	854	0.22	0.25	0.11	0.09
3	542	421	0.15	0.19	0.19	0.17
4	ND	736	0.28	0.26	0.15	0.11
5	799	831	0.13	0.29	0.12	0.05
6	731	569	0.24	0.20	0.07	0.14
7	905	584	0.31	0.29	0.09	0.10
8	823	639	0.20	0.24	0.15	0.11
9	642	614	0.18	0.25	0.11	0.10
10	552	580	0.24	0.19	0.10	0.10
Mean ± SD	702 ± 123	615 ± 139	0.22 ± 0.06	0.23 ± 0.04	0.12 ± 0.03	0.11 ± 0.03

GBM, glomerular basement membrane width; Vv(Mes/glom), volume fraction of mesangium; Sv(PGBM/glom), surface density of peripheral GBM; SD, standard deviation.

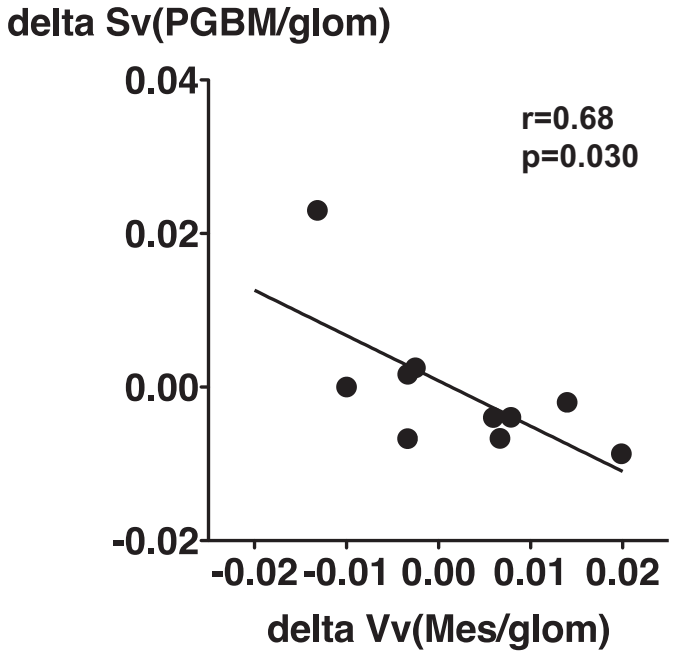

Fig. 1. The relationship between change in Vv(Mes/glom) and change in Sv(PGBM/glom). The annual change in the Sv(PGBM/glom) was negatively correlated with that of the Vv (Mes/glom) ($r = 0.68$, $p = 0.030$) during 6 years of follow-up.

microalbuminuria together were associated with a decline in renal function,[31,32] further and larger studies are required to elucidate whether diabetic retinopathy and nephropathy progress in parallel.

The strength of the present study is that we performed serial renal biopsies to examine the detailed chronological changes in renal histology and function in normo- and microalbuminuric patients with type 2 diabetes. We also used a direct measure of GFR using iohexol clearance. We believe that this is the first report to show that GFR decreases as a result of a decrease in glomerular filtration surface secondary to mesangial expansion in patients with type 2 diabetes and early nephropathy.

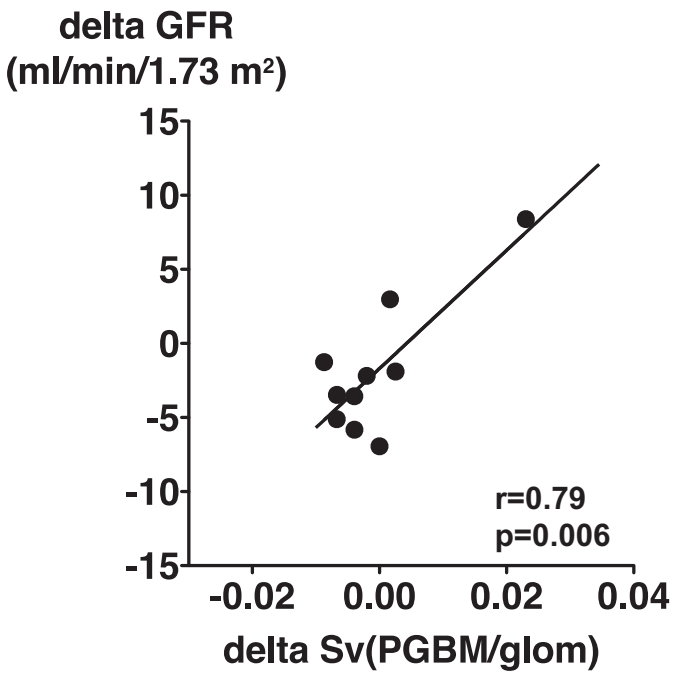

Fig. 2. The relationship between change in GFR and change in Sv(PGBM/glom). The annual decrease in the Sv(PGBM/glom) was positively correlated with the decrease in the GFR over the 6 years after the initial renal biopsy ($r = 0.79$, $p = 0.006$).

The present study is associated with some limitations. First, we were only able to analyze a small number of patients, which might have caused some of our results to not reach statistical significance (e.g. the lack of a significant increase in the UAE and the correlation of changes between the UAE and morphological values). In Japan, research renal biopsies are difficult to perform at early stages of diabetic nephropathy. Another limitation is that we excluded patients with comorbidities and hypertension. This may introduce a significant bias as patients with type 2 diabetes and nephropathy have concomitant hypertension and cardiovascular disease. However, we deliberately set out to explore renal structural-functional relationships in those with early nephropathy. In addition, the GFR for each individual fluctuated considerably over the years. The same phenomenon occurs in UAE. Therefore, single point measurements (baseline and end of follow-up) for each individuals could result in the possibility of an interpretation bias. We should also be careful to interpret the results from the control subjects because their tissues were obtained after and not prior to kidney transplantation.

In conclusion, in normo- and microalbuminuric patients with type 2 diabetes who had a definite histological diagnosis of diabetic nephropathy, a decrease in GFR was associated with a decreased glomerular filtration surface area, which, in turn, reflected mesangial expansion over six years of follow-up. These changes, which represent the initial changes in early diabetic nephropathy, occurred without a worsening of albuminuria or DR. Further studies in a larger group of patients may be warranted to confirm the utility of estimates of glomerular filtration surface area in predicting progression toward end stage renal disease in type 2 diabetes.

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Authors' contributions

TM collected and analyzed the data and developed and wrote the manuscript, including reviewing the literature. TY analyzed the data and contributed to the discussion; MM and MO also contributed to the discussion. TM is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. This study was presented in part at the 77th Annual Scientific Meeting of the American Diabetes Association, San Diego, CA, June 9–13, 2017.

Conflict of interest

All the authors declared no conflicts of interest in association with the present study.

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