The Relationship of Diabetic Retinopathy to Preclinical Diabetic Glomerulopathy Lesions in Type 1 Diabetic Patients

The Renin-Angiotensin System Study

Ronald Klein,[1] Bernard Zinman,[2] Robert Gardiner,[3] Samy Suissa,[4] Sandra M. Donnelly,[5] Alan R. Sinaiko,[6] Michael S. Kramer,[7] Paul Goodyer,[8] Scot E. Moss,[1] Trudy Strand,[6] and Michael Mauer[6]

Few epidemiological data exist regarding the correlation of anatomic measures of diabetic retinopathy and nephropathy, especially early in the disease processes. The aim of this study was to examine the association of severity of diabetic retinopathy with histological measures of diabetic nephropathy in normoalbuminuric patients with type 1 diabetes. The study included participants (n = 285) in the Renin-Angiotensin System Study (RASS; a multicenter diabetic nephropathy primary prevention trial) who were aged ≥16 years and had 2–20 years of type 1 diabetes with normal baseline renal function measures. Albumin excretion rate (AER), blood pressure, serum creatinine, and glomerular filtration rate (GFR) were measured using standardized protocols. Diabetic retinopathy was determined by masked grading of 30° color stereoscopic fundus photographs of seven standard fields using the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale. Baseline renal structural parameters, e.g., fraction of the glomerulus occupied by the mesangium or mesangial fractional volume [Vv(Mes/glom)] and glomerular basement membrane width, were assessed by masked electron microscopic morphometric analyses of research percutaneous renal biopsies. No retinopathy was present in 36%, mild nonproliferative diabetic retinopathy in 53%, moderate to severe nonproliferative diabetic retinopathy in 9%, and proliferative diabetic retinopathy in 2% of the cohort. Retinopathy was not related to AER, blood pressure, serum creatinine, or GFR. All renal anatomical end points were associated with increasing severity of diabetic retinopathy, while controlling for other risk factors. These data demonstrate a significant association between diabetic retinopathy and preclinical morphologic changes of diabetic nephropathy in type 1 diabetic patients. *Diabetes* 54: 527–533, 2005

Epidemiological studies have shown that diabetic retinopathy and nephropathy are closely associated (1–4). Although diabetic retinopathy appears to be more common than nephropathy (90% of patients with ≥20 years of type 1 diabetes compared with 25–40% for those showing signs of nephropathy), this may be misleading (4). Estimates of prevalence of retinopathy are usually based on direct examination of anatomical retinal changes, whereas those of nephropathy are defined by functional abnormalities such as microalbuminuria or overt proteinuria. However, renal anatomical changes, sometimes quite advanced, can also occur in the absence of renal functional abnormalities in patients with type 1 diabetes (5,6). Only a few small studies have examined relationships between the anatomical lesions of retinopathy and nephropathy in patients with diabetes (7–10). The purpose of this report is to describe the association of severity of retinopathy with histological measures of nephropathy in a well-characterized cohort of normotensive patients with type 1 diabetes with normal renal function.

RESEARCH DESIGN AND METHODS

The Renin-Angiotensin System Study (RASS) is a parallel, double-blind, placebo-controlled, multicenter, primary prevention clinical trial of diabetic nephropathy conducted at three clinical centers in Minneapolis, Minnesota, and Montreal and Toronto, Canada. The study design and cohort description have been detailed elsewhere (11). All data were collected with institutional review board approval in conformity with all federal and state laws, and the study was in adherence to the tenets of the Declaration of Helsinki as revised in 1983. Informed consent was obtained. Subjects were aged ≥16 years with

From the [1]Department of Ophthalmology and Visual Sciences, University of Wisconsin Medical School, Madison, Wisconsin; the [2]Division of Medicine, University of Toronto, Toronto, Ontario, Canada; the [3]Division of Medicine, McGill University, Montreal, Quebec, Canada; the [4]Departments of Epidemiology and Biostatistics and Medicine, McGill University, Montreal, Quebec, Canada; the [5]Department of Medicine, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; the [6]Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota; the [7]Departments of Pediatrics and Epidemiology and Biostatistics, McGill University, Montreal, Quebec, Canada; and the [8]Department of Pediatrics, McGill University, Montreal, Quebec, Canada.

Address correspondence to Ronald Klein, MD, MPH, Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, 610 North Walnut St., 460 WARF Madison, WI 53726. E-mail: kleinr@epi.ophth.wisc.edu.

Received for publication 30 July 2004 and accepted in revised form 19 October 2004.

R.K. has received honoraria for serving on the steering committee for the DIRECT Program from Astra-Zeneca. R.G. holds stock in and has received consulting fees from Merck. S.M.D. has received consulting fees from Merck.

AER, albumin excretion rate; ETDRS, Early Treatment Diabetic Retinopathy Study; GBM, glomerular basement membrane; GFR, glomerular filtration rate; RASS, Renin-Angiotensin System Study; Sv(PGBM), surface density of peripheral glomerular capillary GBM; Vv(MC/glom), fractional volume of the glomerulus occupied by mesangial cells.

TABLE 1
Characteristics of the cohort at baseline by fundus photography status

Variable	Fundus photographs	
	Present	Absent
n	252*	32
Age (years)	31.0 ± 9.6	25.4 ± 9.4
Sex (% male)	48	38
Diabetes duration (years)	11.6 ± 4.7	11.3 ± 4.6
HbA_{1c} (%)	8.5 ± 1.6	8.9 ± 1.6
Body mass (kg/m^2)	25.7 ± 3.6	25.4 ± 3.4
Systolic blood pressure (mmHg)	119 ± 10	117 ± 9
Diastolic blood pressure (mmHg)	70 ± 8	69 ± 10
Mean arterial blood pressure (mmHg)	86 ± 8	85 ± 9
AER (μg/min)	6.5 ± 6.0	6.0 ± 5.5
Serum creatinine (mg/dl)	0.81 ± 0.15	0.77 ± 0.11
GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	129 ± 20	128 ± 17
Peripheral GBM surface density/glomerulus	0.13 ± 0.02	0.13 ± 0.02
Mesangial fractional volume/glomerulus	0.21 ± 0.05	0.20 ± 0.04
Mesangial matrix fractional volume/glomerulus	0.12 ± 0.03	0.11 ± 0.03
Vv(MC/glom)	0.075 ± 0.019	0.071 ± 0.020
GBM width (nm)	476 ± 93	497 ± 101
Glomerulopathy index	59.3 ± 10.7	61.0 ± 11.6

Data are means ± SD, unless otherwise noted. *Except for AER, where $n = 251$.

2–20 years of type 1 diabetes and onset before their 45th birthday. All were normotensive, normoalbuminuric (albumin excretion rate [AER] <20 μg/min on at least two of three timed overnight urine collections), and with a normal or increased glomerular filtration rate (GFR; ≥90 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$). A total of 285 subjects were randomized into one of the following three treatment groups: losartan (an angiotensin II blocker), enalapril (an ACE inhibitor), or placebo (11).

Pertinent parts of the baseline examination included measurement of blood pressure, with the participant in the seated position after resting for 5 min, using an automated blood pressure device (vital signs monitor 18465X; DinaMap). Height and weight were measured according to standard anthropometric procedures. Pupils were dilated, and 30° color stereoscopic fundus photographs were taken of the seven standard fields, as defined in the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol (12). The photographs were graded in a masked fashion by the University of Wisconsin Ocular Epidemiology Reading Center using the modified Airlie House classification scheme and the ETDRS retinopathy severity scale. Grading protocols have been described in detail elsewhere (12,13). For each eye, the maximum grade in any of the seven standard photographic fields was determined for each of the lesions and used to define the following "retinopathy levels" (14–16).

- Level 10: No retinopathy.
- Level 21: Microaneurysms only or retinal hemorrhages or soft exudates in the absence of microaneurysms.
- Level 31: Microaneurysms and one or more of the following: venous loops ≥31 μm; questionable soft exudate, intraretinal microvascular abnormalities, or venous beading; or retinal hemorrhage.
- Level 37: Microaneurysms and one or more of the following: hard exudate or soft exudate.
- Level 43: Microaneurysms and one or more of the following: hemorrhages/microaneurysms equaling or exceeding those in standard photo one in four or five fields, hemorrhages/microaneurysms equaling or exceeding those in standard photo 2A in one field, and intraretinal microvascular abnormalities in one to three fields.
- Level 47: Microaneurysms and one or more of the following: both intraretinal microvascular abnormalities and hemorrhage/microaneurysm characteristics from level 43, intraretinal microvascular abnormalities in four or five fields, hemorrhages/microaneurysms equaling or exceeding those in standard photo 2A in two or three fields, or venous beading in one field.
- Level 53: Microaneurysms and one or more of the following: any two or three characteristics from level 47, hemorrhages/microaneurysms equaling or exceeding those in standard photo 2A in four or five fields, intraretinal microvascular abnormalities equaling or exceeding those in standard photo 8A, or venous beading in two or more fields.
- Level 60: Fibrous proliferations only.
- Level 61: No evidence of levels 60 or 65, but scars of photocoagulation either in "scatter" or confluent patches, presumably directed at new vessels.
- Level 65: Proliferative diabetic retinopathy less than Diabetic Retinopathy Study high-risk characteristics, with lesions as follows: new vessels elsewhere, new vessels on or within one disc diameter of the disc graded less than standard photo 10A, or preretinal or vitreous hemorrhage less than one disc area.
- Level 71: Diabetic Retinopathy Study high-risk characteristics, with lesions as follows: vitreous and/or preretinal hemorrhage equaling or exceeding one disc area, new vessels elsewhere equaling or exceeding one-half disc area with vitreous hemorrhage and/or preretinal hemorrhage, new vessels on or within one disc diameter less than standard photo 10A with vitreous hemorrhage and/or preretinal hemorrhage, or new vessels on or within one disc diameter equaling or exceeding standard photo 10A.
- Level 75: Advanced proliferative diabetic retinopathy, with lesions as follows: new vessels on or within one disc diameter equaling or exceeding standard photo 10A with vitreous hemorrhage and/or preretinal hemorrhage.
- Level 85: End-stage proliferative diabetic retinopathy, with lesions as follows: macula obscured by vitreous hemorrhage and/or preretinal hemorrhage, retinal detachment at center of macula, phthisis bulbi, or enucleation secondary to complications of diabetic retinopathy.

For purposes of classification, if the retinopathy severity could not be graded in an eye, it was considered to have a score equivalent to that in the other eye. Retinopathy severity was further divided into four levels: none (level 10), early nonproliferative diabetic retinopathy (levels 21–37), moderate to severe nonproliferative diabetic retinopathy (levels 43–53), and proliferative retinopathy (levels 60–85). The retinopathy grade based on the more advanced eye was used in the analyses.

GFR was estimated at baseline by plasma clearance of nonradioactive iohexol (Omnipaque 300; Shearing). We injected 5 ml iohexol solution intravenously over 2 min, and blood samples were taken from the contralateral arm at 120, 150, 180, 210, and 240 min (±15 min) (17,18). Plasma was stored at −20°C for high-performance liquid chromatography determination of iohexol concentration. Plasma clearance of iohexol was calculated using standardized procedures (17). The AER was measured in timed overnight

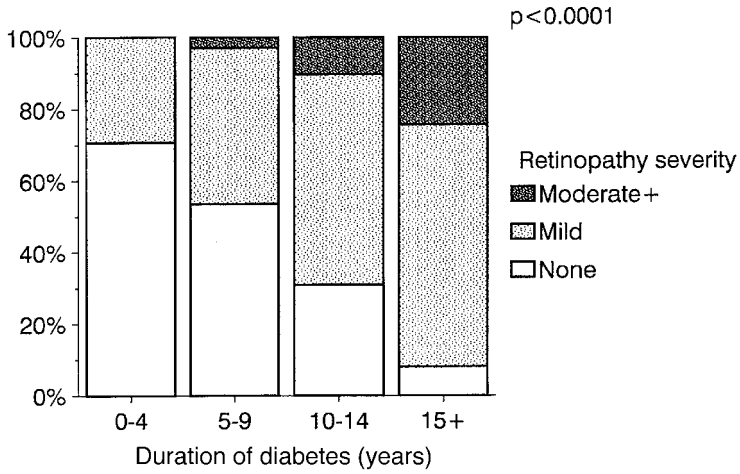

FIG. 1. Distribution of diabetic retinopathy severity by duration of diabetes.

urine collections using a sensitive fluorescence immunoassay (19). The results were expressed in μg/min. Normoalbuminuria was defined as having at least two of three readings of AER being <20 μg/min. HbA_{1c} was measured by high-performance liquid chromatography (Diamat HbA_{1c} analyzer; BioRad, Hercules, CA) using methods utilized by the Diabetes Control and Complications Trial within 3 weeks before kidney biopsy and randomization (20).

Baseline percutaneous renal biopsies were obtained within 6 months before randomization using Franklin-modified Vim-Silverman, Biopty, or Truecut needles. Electron microscopic tissue was fixed in 2.5% glutaraldehyde. After postfixation and embedding, thick sections (1 μm) were stained to select the center-most intact glomerulus in the block for electron microscopy. Ultrathin sections (50–70 nm) were stained with saturated uranyl acetate and lead citrate. Electron microscopic digital images were obtained and stored as TIFF (tagged image file format) files. Adobe Photoshop 6.0 software was used to build montages from the digital images and to superimpose counting grids over the images. An image calibration grid (2,160 lines/mm) was obtained at the beginning of each microscopy session (5). After the center-most glomerulus in a block was randomly entered, 10–20 evenly spaced digital images representing an unbiased systematic sample of ~30% of the glomerular profile was obtained at ×11,000 for measurement of glomerular basement membrane (GBM) width (5,21–23) and mesangial composition (5,24). Images taken at ×3,900 were constructed into a montage of the entire glomerular profile for measurement of the fraction of the glomerulus occupied by the mesangium or mesangial fractional volume [Vv(Mes/glom)], mesangial matrix fractional volume [Vv(MM/glom)], mesangial cell fractional volume [Vv(MC/glom)], and the area of the peripheral GBM filtration surface per glomerulus or surface density of peripheral glomerular capillary GBM [Sv(PGBM)] (5,25).

A glomerulopathy index was computed using the formula described by Rudberg et al. (26): glomerulopathy index = GBM width/10 + [Vv(MM/glom) × 100].

Renal anatomical end points, except Sv(PGBM), were categorized as being normal or abnormal according to whether they were >2 SD above the means of a group of age- and sex-matched nondiabetic control subjects. Abnormal Sv(PGBM) was defined as <2 SD below the mean of the control group.

Statistics. Statistical analyses were conducted using SAS version 8. Means were compared for statistically significant differences by *t* test and ANOVA when two or more than two groups, respectively, were involved. Multivariable associations between renal anatomic characteristics and diabetic retinopathy and other independent variables such as age, sex, duration of diabetes, HbA_{1c}, blood pressure, body mass, white blood cell count, and serum potassium were explored by multiple linear regression. Univariate relationships between renal anatomic characteristics defined as normal or abnormal and diabetic retinopathy were evaluated by the Mantel-Haenszel test of trend (27). Similarly, multivariable relationships, where other independent variables were included, were evaluated by logistic regression.

RESULTS

Of the 285 participants, 1 had insufficient glomeruli for morphometric measurements, 32 had renal biopsy data without gradable fundus photographs, and 252 had both gradable fundus photographs and biopsy data. Subjects with gradable fundus photographs were older than those without gradable fundus photographs ($P = 0.002$); otherwise, there were no statistically significant differences between groups for other characteristics (Table 1).

No diabetic retinopathy was present in 35%, mild nonproliferative diabetic retinopathy in 54%, moderate to severe nonproliferative diabetic retinopathy in 9%, and proliferative retinopathy in 2% of the cohort. The prevalence and severity of retinopathy were associated with longer duration of diabetes (Fig. 1) and higher HbA_{1c} level (Fig. 2). Age, sex, BMI, white blood cell count, and serum

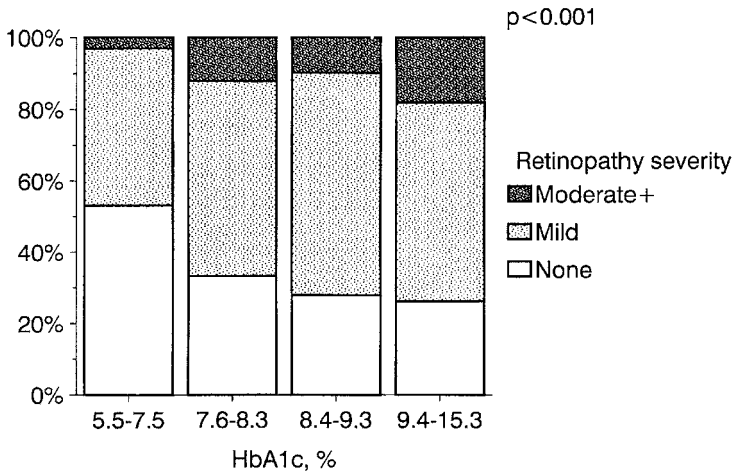

FIG. 2. Distribution of diabetic retinopathy severity by HbA_{1c}.

TABLE 2
Renal functional and structural characteristic by diabetic retinopathy severity

Renal characteristic	Diabetic retinopathy severity			
	No DR	Early NPDR	Moderate to severe NPDR and PDR	*P*
n	89	136	27	
Function				
Systolic blood pressure (mmHg)	118 ± 10	119 ± 10	120 ± 12	0.79
Diastolic blood pressure (mmHg)	70 ± 9	70 ± 7	72 ± 8	0.34
Mean arterial blood pressure (mmHg)	86 ± 8	86 ± 7	88 ± 8	0.46
AER (μm/min)	5.9 ± 4.0	6.5 ± 5.9	8.4 ± 9.9	0.16
Log (AER)	1.55 ± 0.68	1.63 ± 0.70	1.67 ± 0.96	0.66
Serum creatinine (mg/dl)	0.83 ± 0.15	0.80 ± 0.14	0.78 ± 0.14	0.13
GFR ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	128 ± 20	128 ± 19	134 ± 30	0.39
Structure				
GBM width (nm)	430 ± 80	496 ± 83	527 ± 116	<0.0001
Mesangial fractional volume/glomerulus	0.20 ± 0.04	0.21 ± 0.04	0.25 ± 0.05	<0.0001
Mesangial matrix fraction volume/glomerulus	0.11 ± 0.03	0.12 ± 0.03	0.14 ± 0.03	<0.0001
Vv(MC/glom)	0.07 ± 0.02	0.07 ± 0.02	0.09 ± 0.02	0.002
Peripheral GBM surface density/glomerulus	0.134 ± 0.021	0.127 ± 0.020	0.118 ± 0.021	0.001
Glomerulopathy index	53.6 ± 9.1	61.5 ± 9.7	66.8 ± 11.6	<0.0001

Data are means ± SD. DR, diabetic retinopathy; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

potassium levels were not associated with diabetic retinopathy severity.

Diabetic retinopathy severity was not associated with resting mean blood pressure (Table 2) or systolic and diastolic blood pressure, AER, serum creatinine, or GFR (Table 2). Diabetic retinopathy severity was significantly related to each of the renal anatomical end points (Table 2 and Fig. 3). While controlling for other risk factors, including age, diabetes duration, sex, HbA_{1c}, mean blood pressure, and BMI, the association of retinopathy severity with all renal end points other than Vv(MC/glom) and surface density of peripheral capillary GBM per glomerulus [Sv(PGBM/glom)] remained (Table 3).

Univariate analyses showed retinopathy severity to be associated with most renal anatomical end points categorized as being abnormal (Table 4). However, while controlling for HbA_{1c} levels and duration of diabetes, only the associations of retinopathy severity with abnormalities in GBM width and glomerulopathy index values remained statistically significant, while the association with Vv(MC/glom) values was of borderline statistical significance (Table 5). Removal of HbA_{1c} from the multivariate analyses did not change these relationships.

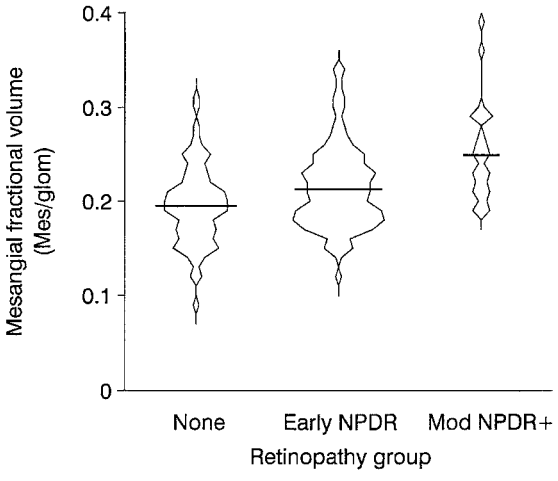

FIG. 3. Spindle plot showing distribution of mesangial fractional volume by diabetic retinopathy severity. The width of each symmetric figure represents the frequency of mesangial fractional volume at the value on the vertical scale. The heavy horizontal lines represent the means. Mod, moderate; NPDR, nonproliferative diabetic retinopathy.

DISCUSSION

RASS provides unique data regarding the association of retinopathy severity with morphologically evaluated preclinical nephropathy in patients with type 1 diabetes. The study uses standardized protocols both for measurement (including objective recording and grading of diabetic retinopathy using stereoscopic fundus photographs of seven standard fields) and for quantitation of glomerular structure, using digitized electronic microscopic morphometric analysis of renal biopsy specimens (5,6,12–16,21–25).

Previous attempts to correlate diabetic retinal with renal changes have been conducted in patients with severe manifestations of these complications (7,8,10,28). Thickening of basement membrane in both retinal and glomerular capillary vessels has been shown in late stages of diabetic retinopathy and nephropathy (9). In 86 patients with type 1 diabetes with more severe disease who were being evaluated for pancreatic transplant alone, all subjects had retinopathy, 70% (60 of 86) of whom had proliferative retinopathy, and 73% (63 of 86) had clinical nephropathy, defined as persistent microalbuminuria or overt proteinuria but without advanced renal insufficiency (GFR $\geq 102 \pm 30\ ml \cdot min^{-1} \cdot 1.73\ m^{-2}$) (2). More severe retinopathy was associated with advanced nephropathy, as defined by increased mesangial fractional volume and decreased peripheral GBM surface density. However, in the few subjects without clinical nephropathy or with minimal microalbuminuria, there was marked discordance between measures of retinopathy and anatomical measures of nephropathy. However, patients with more severe nonrenal complications, such as retinopathy, may have

TABLE 3
Multivariate association of diabetic retinopathy with renal anatomic characteristics controlling for age, duration of diabetes, sex, HbA_{1c}, mean arterial blood pressure, and BMI

Renal characteristic	Diabetic retinopathy severity*		
	Early NPDR	Moderate to severe NPDR and PDR	*P*
n	136	27	
GBM width (nm)	48.0	64.8	<0.0002
Mesangial fractional volume	0.0048	0.0257	0.03
Mesangial matrix fraction volume	0.0067	0.0170	0.04
Mesangial cell fraction volume	−0.0029	0.0063	0.06
Peripheral GBM surface density	−0.0043	−0.0095	0.15
Glomerulopathy index	5.5	8.2	0.0001

*Values represent the change in renal characteristic at the diabetic retinopathy severity level compared to no retinopathy (n = 89). NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

self-selected to be considered for pancreas transplantation alone.

Fewer data exist regarding the relation of retinopathy to anatomic renal changes before the onset of clinical nephropathy. In the present study, with a more robust sample and with a selection of individuals with no clinical nephropathy, we observed less discordance between retinopathy severity and anatomic measures of nephropathy. The associations by multivariate analysis between retinopathy and the structural manifestations of nephropathy were stronger when renal structural measures were used as continuous rather than dichotomous variables (normal or abnormal defined as >2 SDs from the normal mean). This may be because there are wide normal ranges for these renal structural measures (21), so that early on in the disease process, some diabetic patients may be developing renal lesions, but starting from relatively low normal values, they may still be in the normal range at the time of their baseline biopsy (29). Also, relationships of retinopathy and GBM width may have been stronger than with mesangial variables because of the different influences of diabetes duration on these two glomerular structural variables. Thus, GBM width increases appear linear with duration, whereas there is little increase in mesangial fractional volume in the first 10–15 years of type 1 diabetes and a more rapid mesangial functional volume increase thereafter (30). Given the mean diabetes duration of 11.6 ± 4.7 years in the present study cohort, the stronger association with GBM width would be expected. Data from the end of RASS may shed further light on this.

Although less discordance was observed in the current study than in previous studies of patients with more severe disease (7,8,10,28), nearly 50% of the subjects without signs of diabetic retinopathy still had an increased glomerulopathy index. This finding may be because retinopathy was detected by grading of gross anatomic changes seen on stereoscopic fundus photographs, whereas the glomerulopathy index was based on electron microscopic detection of renal changes.

Nonetheless, the strong associations between retinopathy findings and renal biopsy measures could be useful in assessing renal risk in patients with type 1 diabetes. Thus, patients who are normoalbuminuric, despite longstanding type 1 diabetes, are still at risk of progression to microalbuminuria. A number of variables, including baseline AER (5,31,32), HbA_{1c} values (32), increased GFR (31), male sex (32), mean arterial clinic blood pressure (32), ambulatory blood pressure measures (31,33), and ambulatory blood pressure nondipper status (33) have been associated with increased risk of progression from normo- to microalbuminuria. Because microalbuminuric patients have worse nephropathy lesions, on average, than normoalbuminuric patients (5,6,34), the above-listed variables are likely predictive of both increasing AER and renal lesions. The present cross-sectional study suggests that retinopathy findings may also serve this predictive role, but a firm conclusion must await longitudinal study of the RASS cohort. In RASS, we have the unique advantage of prospectively evaluating the relationship of retinopathy status and subsequent changes in renal morphology because all patients will undergo an end-of-study renal biopsy as well as retinal fundus photography. Studies have

TABLE 4
Percentage of abnormal renal anatomic characteristics by diabetic retinopathy severity

Renal characteristic	Diabetic retinopathy severity			
	No DR	Early NPDR	Moderate to severe NPDR and PDR	*P*
n	89	136	27	
Increased GBM width	35 (39.3)	90 (66.2)	21 (77.8)	<0.0001
Increased mesangial fractional volume/glomerulus	11 (12.4)	29 (21.3)	13 (48.2)	0.0002
Increased mesangial matrix fraction volume/glomerulus	32 (36.0)	75 (55.2)	20 (74.1)	0.0001
Increased Vv(MC/glom)	6 (6.7)	5 (3.7)	4 (14.8)	0.48
Decreased peripheral GBM surface density/glomerulus	10 (11.2)	21 (15.4)	9 (33.3)	0.02
Increased glomerulopathy index	40 (44.9)	100 (73.5)	23 (85.2)	<0.0001

Data are *n* (%). DR, diabetic retinopathy; NPDR, nonproliferative DR; PDR, proliferative diabetic retinopathy.

TABLE 5
Multivariate association of diabetic retinopathy with renal structural abnormalities controlling for age, duration of diabetes, sex, HbA_{1c}, mean arterial blood pressure, and BMI

Renal characteristic	Diabetic retinopathy severity		
	Early NPDR	Moderate to severe NPDR and PDR	*P*
n	136	27	
Increased GBM width	2.68 (1.36–5.29)	3.94 (1.14–13.62)	0.01
Increased mesangial fractional volume/glomerulus	1.12 (0.48–2.63)	2.31 (0.72–7.48)	0.27
Increased mesangial matrix fraction volume/glomerulus	1.41 (0.73–2.75)	1.74 (0.55–5.58)	0.52
Increased Vv(MC/glom)	0.21 (0.05–0.89)	0.57 (0.10–3.45)	0.06
Decreased peripheral GBM surface density/glomerulus	0.96 (0.38–2.44)	1.90 (0.51–7.06)	0.42
Increased glomerulopathy index	2.51 (1.27–4.95)	3.66 (0.96–14.02)	0.02

Data are odds ratio (95% CI). The no retinopathy group ($n = 89$) is the reference group for the odds ratios. NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

shown that there is overlap between glomerular structural parameters and albuminuria categories (normoalbuminuria, microalbuminuria, and proteinuria) among patients with long-standing type 1 diabetes (5,6). Moreover, normoalbuminuric patients with >15 years of type 1 diabetes may progress to microalbuminuria or proteinuria over the subsequent 10 years of follow-up, and similar outcomes have been seen in patients with ~10 years' duration of type 2 diabetes (35,36). GBM width is greater in microalbuminuric type 1 diabetic patients that progress to proteinuria compared with those who do not (37). Whether glomerular structural characteristics or AER is a more precise predictor of diabetic nephropathy risk, whether the combination is more helpful than either alone, and whether retinopathy status could help in this prediction remains to be determined. Retinopathy, however, was not related to AER in our study. This was not surprising because others have found no correlation between retinopathy severity and urinary albumin excretion in normoalbuminuric patients with type 1 diabetes (38).

In summary, we found cross-sectional associations of retinopathy severity with renal anatomic measures in type 1 diabetic normotensive patients with preclinical nephropathy. The associations of these measures over time may provide further understanding of the relationship of the pathophysiological events responsible for these very common microvascular complications.

ACKNOWLEDGMENTS

These studies were funded by National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Disease Grant DK51975; Merck; Merck Frosst, Canada; and Canadian Institutes of Health Research, Canada, Grant DCT-14281. The University of Minnesota General Clinical Research Center (GCRC) is supported by National Institutes of Health Grant M01-RR-00400. R.K. is supported in part by the Research to Prevent Blindness Senior Scientific Award.

We thank the dedicated staff of the RASS trial in Minneapolis: J. Basgen, Morphometry Lab supervisor; J. Bucksa, Central Biochemistry Lab manager; B. Chavers, Central Albumin Lab director; M. Cohen, fundus photographer; T. Groppoli, electron microscopist; P. Herbert, Morphometry Lab senior technician; K. Johnson, pharmacist; S. Kupcho, Central Albumin Lab supervisor; B. Lohr, pharmacy clinical specialist; D. Luke, pharmacy coordinator; M. Nowicki, Central Laboratory lead technician; J. Pederson, assistant trial coordinator; S. Rozen, electron microscopist; K. Sawyer, Central Albumin Lab junior scientist; P. Stanaitis, fundus photographer; and J. Stein, assistant project manager. We also thank the GCRC staff: Montreal: D. Laforte, trial coordinator; G. Carro-Ciampi, pharmacy coordinator; L. Marcon, fundus photographer; B. Maruca, research assistant; and A. Roy, research nurse. Toronto: A. Barnie, trial coordinator; K. Bowyer, research nurse; A. Roode, research nurse; E. Vivero, research nurse; and L. Tuason, administrative assistant. Montreal Data Center: D. Gaudreau, administrative assistant; C. Hebert, coordinator; V. Lucas, data entry; and M. Senecal and J.A.C. Delaney, statisticians. Finally, we thank the Madison Ocular Epidemiology Reading Center: Tiffany Jan, coordinator; Stacy M. Meuer, Carol J. Hoyer, Maria K. Swift, Andrew Ewen, Ellen R. Hall, and Anne Mosher, photograders; and Shane Hubler, information systems.

REFERENCES

1. Nilsson SV, Nilsson JE, Frostberg N, Emilsson T: The Kristianstad survey. II. Studies in a representative adult diabetic population with special reference to comparison with an adequate control group. *Acta Med Scand* 469 (Suppl.):1–42, 1967
2. Kornerup T: Studies in diabetic retinopathy: an investigation of 1,000 cases of diabetes. *Acta Med Scand* 153:81–101, 1955
3. Knowler WC, Bennett PH, Ballintine EJ: Increased incidence of retinopathy in diabetics with elevated blood pressure: a six-year follow-up study in Pima Indians. *N Engl J Med* 302:645–650, 1980
4. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. V. Proteinuria and retinopathy in a population of diabetic persons diagnosed prior to 30 years of age. In *Diabetic Renal-Retinal Syndrome*. Vol. 3. Friedman EA, L'Esperance FA, Eds. New York, Grune & Stratton, 1986, p. 245–264
5. Fioretto P, Steffes MW, Mauer M: Glomerular structure in non-proteinuric IDDM patients with various levels of albuminuria. *Diabetes* 43:1358–1364, 1994
6. Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, Russell G, Mauer M: Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. *Diabetes* 51:506–513, 2002
7. Chavers BM, Mauer SM, Ramsay RC, Steffes MW: Relationship between retinal and glomerular lesions in IDDM patients. *Diabetes* 43:441–446, 1994
8. 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* 13:2547–2552, 1998
9. Carlson EC: Scanning and transmission electron microscopic studies of normal and diabetic acellular glomerular and retinal microvessel basement membranes (Review). *Microsc Res Tech* 28:165–177, 1994

10. Kanauchi M, Kawano T, Uyama H, Shiiki H, Dohi K: Discordance between retinopathy and nephropathy in type 2 diabetes. *Nephron* 80:171–174, 1998
11. Mauer M, Zinman B, Gardiner R, Drummond KN, Suissa S, Donnelly SM, Strand TD, Kramer MS, Klein R, Sinaiko AR: ACE-I and ARBs in early diabetic nephropathy. *J Renin Angiotensin Aldosterone Syst* 3:262–269, 2002
12. Early Treatment Diabetic Retinopathy Study Research Group: Grading diabetic retinopathy from stereoscopic color fundus photographs: an extension of the modified Airlie House classification: ETDRS report no. 10. *Ophthalmology* 98 (Suppl. 5):786–806, 1991
13. Klein R, Klein BE, Magli YL, Brothers RJ, Meuer SM, Moss SE, Davis MD: An alternative method of grading diabetic retinopathy. *Ophthalmology* 93:1183–1187, 1986
14. Klein BE, Davis MD, Segal P, Long JA, Harris WA, Haug GA, Magli YL, Syrjala S: Diabetic retinopathy: assessment of severity and progression. *Ophthalmology* 91:10–17, 1984
15. Early Treatment Diabetic Retinopathy Study Research Group: Fundus photographic risk factors for progression of diabetic retinopathy: ETDRS report number 12. *Ophthalmology* 98 (Suppl. 5):823–833, 1991
16. Klein R, Klein BE, Moss SE, Cruickshanks KJ: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XIV. Ten-year incidence and progression of diabetic retinopathy. *Arch Ophthalmol* 112:1217–1228, 1994
17. Gaspari F, Perico N, Matalone M, Signorini O, Azzollini N, Mister M, Remuzzi G: Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease. *J Am Soc of Nephrol* 9:310–313, 1998
18. Gaspari F, Perico N, Remuzzi G: Application of newer clearance techniques for the determination of glomerular filtration rate. *Curr Opin Nephrol Hypertens* 7:675–680, 1998
19. Chavers BM, Simonson J, Michael AF: A solid phase fluorescent immunoassay for the measurement of human urinary albumin. *Kidney Int* 25:576–578, 1984
20. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
21. Steffes MW, Barbosa J, Basgen JM, Sutherland DE, Najarian JS, Mauer SM: Quantitative glomerular morphology of the normal human kidney. *Lab Invest* 49:82–86, 1983
22. Jensen EB, Gundersen HJ, Østerby R: Determination of membrane thickness distribution from orthogonal intercepts. *J Microsc* 115:19–33, 1979
23. Brito PL, Fioretto P, Drummond K, Kim Y, Steffes MW, Basgen JM, Sisson-Ross S, Mauer M: Proximal tubular basement membrane width in insulin-dependent diabetes mellitus. *Kidney Int* 53:754–761, 1998
24. Steffes MW, Bilous RW, Sutherland DE, Mauer SM: Cell and matrix components of the glomerular mesangium in type I diabetes. *Diabetes* 41:679–684, 1992
25. Ellis EN, Steffes MW, Goetz FC, Sutherland DE, Mauer SM: Glomerular filtration surface in type I diabetes mellitus. *Kidney Int* 29:889–894, 1986
26. Rudberg S, Osterby R, Dahlquist G, Nyberg G, Persson B: Predictors of renal morphological changes in the early stage of microalbuminuria in adolescents with IDDM. *Diabetes Care* 20:265–271, 1997
27. Mantel N: Chi-square tests with one degree of freedom: extensions of the Mantel-Haenszel procedure. *J Am Stat Assoc* 58:690–700, 1963
28. Williamson JR, Tilton RG, Chang K, Kilo C: Basement membrane abnormalities in diabetes mellitus: relationship to clinical microangiopathy (Review). *Diabetes Metab Rev* 4:339–370, 1988
29. 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* 312:1282–1287, 1985
30. Drummond KN, Kramer MS, Suissa S, Levy-Marchal C, Dell'Aniello S, Sinaiko A, Mauer M, International Diabetic Nephropathy Study Group: The effects of duration and age at onset of type 1 diabetes on preclinical manifestations of nephropathy. *Diabetes* 52:1818–1824, 2003
31. Dahlquist G, Stattin EL, Rudberg S: Urinary albumin excretion rate and glomerular filtration rate in the prediction of diabetic nephropathy: a long-term follow-up study of childhood onset type-1 diabetic patients. *Nephrol Dial Transplant* 16:1382–1386, 2001
32. Hovind P, Tarnow L, Rossing P, Jensen BR, Graae M, Torp I, Binder C, Parving HH: Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study. *BMJ* 328:1105, 2004
33. Lurbe E, Redon J, Kesani A, Pascual JM, Tacons J, Alvarez V, Batlle D: Increase in nocturnal blood pressure and progression to microalbuminuria in type 1 diabetes. *N Engl J Med* 347:797–805, 2002
34. Walker JD, Close CF, Jones SL, Rafftery M, Keen H, Viberti G, Osterby R: Glomerular structure in type-1 (insulin-dependent) diabetic patients with normo- and microalbuminuria. *Kidney Int* 41:741–748, 1992
35. Forsblom CM, Groop PH, Ekstrand A, Tötterman KJ, Sane T, Saloranta C, Groop L: Predictors of progression from normoalbuminuria to microalbuminuria in NIDDM. *Diabetes Care* 21:1932–1938, 1998
36. Steffes MW, Chavers BM, Bilous RW, Mauer SM: The predictive value of microalbuminuria. *Am J Kidney Dis* 13:25–28, 1989
37. Bangstad HJ, Østerby R, Hartmann A, Berg TJ, Hanssen KF: Severity of glomerulopathy predicts long-term urinary albumin excretion rate in patients with type 1 diabetes and microalbuminuria. *Diabetes Care* 22:314–319, 1999
38. Poulsen PL, Bek T, Ebbehloh E, Hansen KW, Mogensen CE: 24-h ambulatory blood pressure and retinopathy in normoalbuminuric IDDM patients. *Diabetologia* 41:105–110 1998