Is Diabetic Nephropathy Inherited?

Studies of Glomerular Structure in Type 1 Diabetic Sibling Pairs

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Only a minority of patients with type 1 diabetes develop diabetic nephropathy (DN). Poor glycemic control cannot fully explain DN risk, and family studies suggest genetic susceptibility factors. To understand familial DN concordance, we evaluated glomerular structure in families with type 1 diabetic sibling pairs. Kidney function and biopsy studies were performed in 21 probands (P) (first to develop diabetes) and 21 siblings (S) (second to develop diabetes), most with normal urinary albumin excretion rates (UAER). Glomerular structure was measured by morphometry. Intrafamilial correlation was estimated by one-way random-effects ANOVA and by mixed-effects ANOVA, adjusting for age and duration of diabetes. Diabetes duration was, by definition, longer in P than in S, while age and sex were similar. HbA_{1c} over 5 years and blood pressure were not different in P and S and were without familial effect. UAER was greater in P than in S ($P < 0.05$), with strong familial effect ($P = 0.03$). A strong concordance among siblings for mesangial fractional volume ($P \leq 0.01$) remained significant after adjustment for diabetes duration and age ($P = 0.04$). Results were similar for mesangial cell ($P = 0.01$; adjusted $P = 0.04$) and mesangial matrix fractional volumes ($P < 0.01$; adjusted $P = 0.06$). There was also clustering of the patterns of glomerular lesions. For example, if P had relatively marked glomerular basement membrane thickening compared with mesangial matrix expansion, S had a similar pattern (χ^2, $P < 0.025$). Strong concordance in severity and patterns of glomerular lesions in type 1 diabetic siblings, despite lack of concordance in glycemia, supports an important role for genetic factors in DN risk. ***Diabetes*** **48:865–869, 1999**

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ANOVA, analysis of variance; CCB, calcium channel blocker; CRC, Clinical Research Center; DN, diabetic nephropathy; GBM, glomerular basement membrane; GFR, glomerular filtration rate; P, proband; S, sibling; UAER, urinary albumin excretion rate; Vv(MC/glom), mesangial cell fractional volume; Vv(Mes/glom), mesangial fractional volume; Vv(MM/glom), mesangial matrix fractional volume.

Clinical diabetic nephropathy (DN) results from glomerular, tubular, interstitial, and vascular lesions (1) that achieve levels of severity where normal glomerular permselectivity, filtration function, or blood pressure regulation are no longer possible.

Only a minority of patients with type 1 diabetes develops overt DN and progress toward end-stage renal disease (2,3). Epidemiologic studies and intervention trials have shown that poor glycemic control is an important risk factor for DN (4–6), but it is clear that glycemia, per se, cannot explain all of the variability in DN risk. Natural history studies of DN have indicated increasing incidences of proteinuria in type 1 diabetic patients in ascending quartiles of hyperglycemia (3). Nonetheless, some patients in the best control quartile developed proteinuria, whereas the majority in the worst quartile escaped this complication (3). Other environmental factors such as smoking (7) explain only a fraction of the residual variability in risk, and the variability was largely mysterious until Seaquist et al. (8) described a strong familial concordance for DN risk among type 1 diabetes multiplex families. Only 17% of the diabetic siblings of 26 diabetic renal transplant recipients escaped DN, while for 12 probands with ≥20 years of diabetes duration who lacked clinical findings of renal disease, only 17% of their diabetic siblings had urinary albumin excretion rates (UAERs) >45 mg/24 h (8). This work was largely confirmed by type 1 diabetic sibling studies of Borch-Johnsen et al. (9) and Quinn et al. (10). Familial clustering of renal disease has also been reported among type 2 diabetic Pima Indians (parent-child concordance) (11) as well as among type 2 diabetic African-American relatives (12), both ethnic groups established as having considerably higher incidences of DN than Caucasian populations (11–13).

In this study, we examined the lesions of DN among type 1 diabetic siblings and determined that there was strong concordance for the severity and the patterns of glomerular lesions among the sibling pairs. We conclude that familial concordance in lesions accounts, at least in part, for the familial concordance in DN risk.

RESEARCH DESIGN AND METHODS

Patients were recruited from a midwestern U.S. registry of type 1 diabetic patients. Eligible for the study were adult type 1 diabetic patients with one or more type 1 diabetic siblings. Siblings with diabetes duration of at least 10 years were asked to participate. One family in which siblings had diabetes durations of 6 and 5 years also wished to participate and were included. Eighty-three eligible sibling pairs were contacted, and 21 pairs agreed to participate. The 42 patients all provided informed consent for studies of renal structure and function and glycemic

TABLE 1
Demographic, clinical, and renal functional data in the type 1 diabetic sibling pairs

	n	Age (years)	Diabetes duration (years)	HbA_{1c} (%)	UAER (μg/min)	Creatinine clearance ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	Mean blood pressure (mmHg)
Probands	21	36 ± 8	25 ± 11	8.5 ± 0.9	7 (2–2,589)	102 ± 25	88.7 ± 9.8
Siblings	21	37 ± 8	17 ± 8	8.8 ± 1.5	5 (1–1,334)	103 ± 24	84.9 ± 6.9
P values	—	NS	ND	NS	<0.05	NS	NS

Data are means ± SD except UAER values, which are expressed as median (range) and were logarithmically transformed before analysis. *P* values were determined by paired *t* test. ND, not done because different by design.

control, and all procedures in this study were approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota. The first patient in each pair to develop type 1 diabetes was called proband (P); the second, sibling (S). There was no selection for patients with diabetic complications, and all willing sibling pairs meeting the above criteria were accepted. Patients with advanced DN (serum creatinine >2.0 mg/dl) were not enrolled, however, since previous studies showed that renal pathology among such patients would approach that of end-stage renal disease.

Controls for the structural data were 60 normal kidney transplant donors matched for age and sex with the diabetic patients (25 men, 35 women, 36 ± 8 years of age).

Study protocol. Patients were admitted to the Clinical Research Center (CRC) at the University of Minnesota for 3 days of testing. UAER was determined by fluorescent immunoassay on three carefully performed sterile overnight urine collections (microalbuminuria 20–200 μg/min; overt proteinuria >200 μg/min). Glomerular filtration rate (GFR) was estimated by creatinine clearance using these urine samples, with plasma and urine creatinine concentrations measured using a modification of the Jaffé reaction (normal values: 90–130 $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$). Blood pressure data are presented as the means of multiple determinations obtained by the CRC nurses during the baseline admission. Seven patients were hypertensive. Six (5 P, 1 S) of the 42 patients were receiving antihypertensive medications, and these drugs were not discontinued during the studies. The medications included a calcium channel blocker (CCB) in two patients, an ACE inhibitor in two patients, a CCB and ACE inhibitor in one patient, and a β-blocker in one patient. HbA_{1c} was measured by high-performance liquid chromatography in a single laboratory (normal values 4.0–6.5%). The mean of multiple values obtained over a 5-year period for each patient was used (median 6 measurements per patient; range 2–16).

Tissue processing and morphometric analysis. Renal tissue was obtained by percutaneous biopsy under ultrasound guidance in diabetic patients and by intraoperative renal biopsy of normal renal transplant donors in controls and was processed for light and electron microscopy (14). Morphometric measurements were performed by a single observer who was unaware of the patients' identities. Tissue for electron microscopy was fixed in 2.5% glutaraldehyde in Millonig's buffer and embedded in Polybed 812. Sections (1 μm thick) were cut and stained with toluidine blue to permit random selection of the centermost, intact glomeruli at least one tubular diameter from the edge of the tissue. When a glomerulus was not present in the first section, another section was observed every 50 μm deeper in the block until the appearance of a glomerulus. At least three nonsclerotic glomeruli per biopsy were photographed with a JEOL 100CX electron microscope at a final magnification of 3,900× to produce photomontages of the entire glomerular profile, defined as the circumscribed, minimal convex polygon enclosing the glomerular tuft (15,16). The montages were used to estimate mesangial fractional volume [Vv(Mes/glom)] by counting the number of fine points falling on mesangium in relation to the number of coarse points hitting the glomerular tuft (15,16); 213 ± 55 (mean ± SD) coarse and 283 ± 111 fine points were counted per biopsy. Another set of micrographs was photographed at a final magnification of ×12,000 by entering the glomerulus at its lowest segment and systematically sampling about 20% of the glomerular profile to estimate glomerular basement membrane (GBM) width by the orthogonal intercept method (17); 116 ± 26 measurements per biopsy were performed. Fractional volumes of mesangial matrix per glomerulus [Vv(MM/glom)] and mesangial cell per glomerulus [Vv(MC/glom)] were estimated by the point-counting technique on the high-magnification photographs (18,19).

Statistical analysis. Data are expressed as mean ± SD; however, UAER values, not normally distributed, are presented as median and range and were logarithmically transformed before analysis. Since the logarithm transformation is monotonic, order was maintained while achieving a normal distribution for statistical analyses.

Demographic and renal functional characteristics of the sibling pairs were compared by paired Student's *t* test. Mixed-effects analysis of variance (ANOVA) was performed to test for an overall difference in the mean values of renal structural parameters between siblings and normal controls. This analysis accounted for the high correlation within sibling pairs for renal structural parameters. Within the sample of sibling pairs, one-way random-effects ANOVA was used to estimate the unadjusted intrafamilial correlation. Subsequently, since siblings were often nearly the same age and frequently demonstrated similar duration of diabetes, the effects of age, age^2, duration, and $duration^2$ were entered into a mixed-effects ANOVA to obtain estimates of intrafamilial correlation adjusted for these factors. Likelihood ratio tests were used to test for the contribution of a familial component to the total variability by comparing models with and without a random family effect. PROC MIXED of the SAS package was used to perform all mixed model analyses.

Concordance of patterns of glomerular structural parameters among sibling pairs was determined as follows: the two structural parameters under consideration [for example, GBM width and Vv(MM/glom)] were plotted along with the regression line describing the relationship of the two parameters. Each patient was represented as a point that was either above or below the regression line. The probability that sibling pairs were concordant—both above or both below the regression line—or discordant was estimated by χ^2 analysis, with values for $P < 0.05$ considered as significant.

RESULTS

Demographics and renal function. Age was similar in P and S groups (Table 1), as was sex distribution (9 men and 12 women in both groups). Diabetes duration was, by definition, longer in the probands than in their respective siblings

TABLE 2
Morphometric measures of glomerular structure in the type 1 diabetic sibling pairs

	n	GBM width (nm)	Vv(Mes/glom)	Vv(MC/glom)	Vv(MM/glom)
Probands	21	502 ± 149	0.34 ± 0.09	0.09 ± 0.03	0.21 ± 0.07
Siblings	21	508 ± 104	0.33 ± 0.10	0.08 ± 0.02	0.20 ± 0.08
P values		NS	NS	NS	NS

Data are means ± SD. *P* values were determined by paired *t* test.

TABLE 3
ANOVA for familial impact

	One-way random-effects ANOVA			Mixed-effects ANOVA		
	Unadjusted intraclass correlation	χ^2 (1 df)	P value	Adjusted intraclass correlation	χ^2 (1 df)	P value
Clinical and renal function values						
HbA_{1c} (%)	0.126	0.22	0.64	0.000	0.00	1.00
UAER (μg/min)	0.438	4.45	0.03	0.422	7.65	0.01
Mean blood pressure (mmHg)	0.000	0.00	1.00	0.000	0.00	1.00
Creatinine clearance ($ml \cdot min^{-1} \cdot 1.73\ m^{-2}$)	0.427	3.81	0.05	0.370	2.15	0.14
Renal structure values						
GBM width (nm)	0.548	6.94	<0.01	0.546	2.62	0.11
Vv(Mes/glom)	0.699	13.37	<0.01	0.586	4.41	0.04
Vv(MC/glom)	0.523	6.18	0.01	0.489	4.11	0.04
Vv(MM/glom)	0.700	13.41	<0.01	0.571	3.62	0.06

χ^2 was obtained from the likelihood ratio test for random family effect; P value tests were for familial component of total variability.

(Table 1). Mean HbA_{1c} over 5 years of observation was similar in P and S (Table 1). UAER values were lower in S than in P ($P < 0.05$) (Table 1). Three probands and 2 siblings had overt proteinuria and 5 P and 1 S had microalbuminuria, but the large majority (31 patients) were normoalbuminuric. Despite this, there was a strong familial concordance for UAER that became more statistically significant after adjusting for age and duration (Table 3). Creatinine clearance as well as systolic (118 ± 13 and 113 ± 10 mmHg in P and S, respectively), diastolic (74 ± 9 and 72 ± 8 mmHg in P and S, respectively), and mean blood pressure (Table 1) were similar in P and S. There was no familial correlation for mean HbA_{1c}, mean blood pressure, or creatinine clearance, especially after adjustment for age and duration (Table 3).

Glomerular structure. Despite the fact that most patients in this study were normoalbuminuric, renal structural abnormalities were present in the diabetic patients compared with the normal controls. GBM width (331 ± 45 nm in controls), Vv(Mes/glom) (0.20 ± 0.03 in controls), and Vv(MM/glom) (0.09 ± 0.02 in controls) were greater in P and S compared with normal controls ($P < 0.001$ for each), while Vv(MC/glom) (0.09 ± 0.02 in controls) was similar in diabetic patients and controls (NS for each). All glomerular structural parameters were remarkably similar in P and S (Table 2). Tests for a familial component to the total phenotypic variability were significant at the 0.05 level for all renal structure variables [GBM width, Vv(Mes/glom), Vv(MC/glom), and Vv(MM/glom)] (Table 3). After adjustment for age, age^2, duration of diabetes, and duration^2, all intrafamilial correlations were reduced. Noticeably, tests for the familial component of the total variance were reduced, approaching statistical significance for Vv(MM/glom) ($P = 0.06$), but Vv(Mes/glom) ($P = 0.04$) and Vv(MC/glom) ($P = 0.04$) remained statistically significant after adjustment (Table 3). By ordering sibling pairs along the horizontal axis according to their mean value for Vv(Mes/glom) (Fig. 1), the positive intrafamilial correlation is seen in the narrower range of values in any of the sibling pairs when compared with the range across all the sibling pairs. Although the familial component of variance for GBM was not statistically significant ($P = 0.11$), the intrafamilial correlation for GBM continued to be large (0.546) (Table 3).

There was also concordance in the patterns of glomerular lesions in sibling pairs. Thus, there was concordance in the patterns of the relationships between GBM width and Vv(MM/glom) within sibling pairs ($\chi^2 = 5.7$, $P < 0.025$). Similarly, concordance was found in the patterns of the relationships within sibling pairs between Vv(MM/glom) and Vv(MC/glom) ($\chi^2 = 5.6$, $P < 0.025$).

DISCUSSION

Two studies (9,10) have largely confirmed the insightful observations of Seaquist et al. describing strong concordance among type 1 diabetic sibling pairs for DN risk, as defined by renal functional parameters (8). Depending on patient selection criteria and definitions for nephropathy, the odds ratios for clinical nephropathy or microalbuminuria in three studies (8–10) ranged from 2.4 to 24 in siblings of probands with nephropathy compared with siblings of probands without nephropathy .

The present study demonstrates strong concordance in diabetic glomerular lesions among sibling pairs with long-term type 1 diabetes and supports the hypothesis of genetic susceptibility to DN. The clustering of glomerular structure in sibling pairs was not associated with similarities in systemic blood pressure or long-term metabolic control, but was, in part, related to similar diabetes duration within families. However, glomerular structural parameters remained concordant among siblings after adjusting for diabetes duration and age. Thus, independent of duration, long-term metabolic control, and blood pressure, sibship was the strongest predictor of diabetic glomerular structural changes. The structural parameters most closely related in siblings were Vv(Mes/glom) and Vv(MC/glom), while, after factoring for duration, there was marginal statistical significance for concordance in Vv(MM/glom).

The strongest familial impact in this present study was on mesangial fractional volume, the structural parameter most closely correlated with renal functional abnormalities in

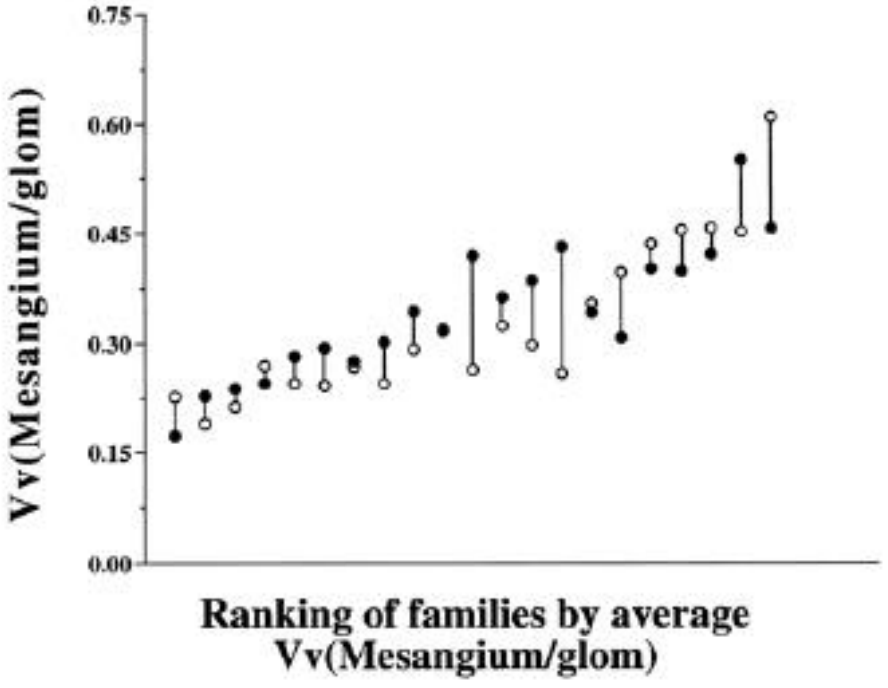

FIG. 1. Vv(Mes/glom) according to sibling pairs. The sibling pairs were ranked by the mean value for Vv(Mes/glom) within the pair: ●, proband; ○, sibling.

cross-sectional studies (1) and the only structural change associated with increasing UAER and clinical progression in longitudinal studies of type 1 diabetic patients (19). There was also strong evidence for clustering of UAER, which was surprising because most patients were normoalbuminuric. It is known that within any functional category (normoalbuminuria, microalbuminuria, or proteinuria) the ranges of glomerular structural abnormalities in patients with type 1 diabetes are quite wide (1,15). For example, renal structure among normoalbuminuric patients can vary from normal to severe lesions bordering on those regularly associated with overt DN (1,15). Thus, concordance among sibling pairs within a given functional category would not per se explain the concordance in structure. Moreover, only five patients were overtly proteinuric, and no patient had serum creatinine values >1.5 mg/dl. Therefore, the current results were not mainly driven by patients with markedly advanced structural and functional alterations. There was concordance in diabetes duration among the sibling pairs, and diabetes duration is directly, albeit imprecisely, related to the severity of DN lesions (1,20). However, important structural relationships among siblings remained after factoring for duration and age.

We found no correlation in long-term glycemic control among the sibling pairs in this study, although other authors have found some concordance when studying larger cohorts of sibling pairs (9,21). Moreover, as discussed below, metabolic control cannot readily explain the concordance in the patterns of glomerular lesions. There is evidence, however, that poor glycemic control is an important risk factor for DN (3–6,22), and there may be levels of hyperglycemia below which nephropathy risk is quite low (6). Findings that genetic factors, as reflected by increased red blood cell sodium/lithium countertransport activity, are more likely to be associated with increased DN risk in patients with poor metabolic control suggest that complex interrelationships of metabolic and genetic variables determine renal responses to the diabetic state (22,23).

Cross-sectional analyses of large numbers of type 1 diabetic patients demonstrated that the correlation between GBM width and Vv(Mes/glom) is inexact ($r = 0.5$) (1). Thus, some patients may have much more thickening of the GBM than mesangial expansion or the contrary. GBM width and Vv(MM/glom) as well as Vv(MC/glom) and Vv(MM/glom) were concordant in pattern among the sibling pairs. Thus, for example, if the proband had relatively marked increase in GBM width for the extent of mesangial expansion, the sibling was likely to have the same pattern. This clustering in patterns of renal lesions was not associated with concordance in metabolic control and may result from genetic variability in the responses of different glomerular cell types to the same environmental disturbances. Indeed, GBM and mesangial matrices probably result from the unique synthetic functions of glomerular mesangial, epithelial, and endothelial cells and probably subsume specific structural and functional requirements of these glomerular regions. Diabetes exerts different influences on these matrix structures; thus there is increased density of α3(IV) and α4(IV) collagen chains in the GBM and decreased α1(IV) and α2(IV) collagen chain density in the mesangium in type 1 diabetic patients with advanced glomerular lesions (24,25). These findings provide a biochemical basis for the heterogeneity of diabetic glomerular lesions as well as the results reported here.

The current results are in keeping with the arguments of Quinn et al. (10) deriving from their studies of DN risk among type 1 diabetic sibling pairs. They reviewed models for environmental and genetic risk factors and concluded that the magnitude of the familial clustering noted in their and other studies could not be explained by environmental concordances alone and were best explained by an autosomal dominant mode of risk inheritance (10). While current data do not directly address inheritance of DN risk, they do suggest that aggregation of renal lesions among sibling pairs represents an

earlier, perhaps genetically determined, point in the pathway to DN. Further, although numbers of pairs were insufficient for formal analysis, the apparently narrower ranges of variation in Vv(Mes/glom) within sibling pairs at the extremes of the distribution in Fig. 1 supports the single major gene effect concept (26).

In summary, although shared environmental risk factors cannot be completely excluded, the remarkable concordance in severity and patterns of glomerular lesions found among type 1 diabetic sibling pairs supports genetic similarities operating through the regulation of complex glomerular cellular responses to diabetes. Although work to date has not been conclusive (27), our findings support the need for further studies to identify genes linked to risk of DN.

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