The Early Natural History of Nephropathy in Type 1 Diabetes

III. Predictors of 5-Year Urinary Albumin Excretion Rate Patterns in Initially Normoalbuminuric Patients

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Predictors of albumin excretion rate (AER) abnormalities could provide earlier indicators of diabetic nephropathy risk. Data from the Natural History Study, a prospective 5-year observation of renal structure and function in young type 1 diabetic patients, were examined for predictors of AER patterns in normoalbuminuric type 1 diabetic patients. Included were 170 patients (96 females) (aged 16.7 ± 5.9 years, duration of diabetes 8.0 ± 4.3 years) with normal blood pressure, normoalbuminuria (AER <20 µg/min), and eight or more follow-up visits over 5 years. AER, blood pressure, and HbA$_{1c}$ (A1C) were determined quarterly and glomerular filtration rate (GFR) annually. Persistent microalbuminuria (PMA) was defined as 20–200 µg/min in two of three consecutive values within 6–12 months. Four different AER patterns were identified. Group 1 (n = 99): all values <20 µg/min. Group 2 (n = 49): intermittent levels >20 µg/min but not meeting microalbuminuria criteria. Group 3 (n = 14): PMA during follow-up but normoalbuminuria at study exit. Group 4 (n = 8): microalbuminuria at study exit. Group 4 (497 ± 95 nm, P < 0.01) and group 3 (464 ± 113 nm, P = 0.03) patients had greater baseline glomerular basement membrane (GBM) width versus group 1 (418 ± 67 nm). Baseline GFR in group 4 (163 ± 37 ml · min^{-1} · 1.73 m^{-2}) was higher than group 1 (143 ± 28 ml · min^{-1} · 1.73 m^{-2}, P = 0.04). A1C was higher in group 2 (9.0 ± 1.2%) than group 1 (8.4 ± 1.1%, P = 0.008). Thus, greater increases in GBM width and GFR were predictors of PMA. Since 64% of the patients that developed microalbuminuria reverted to normoalbuminuria, the risk of diabetic nephropathy as defined by current microalbuminuria criteria is unclear. *Diabetes* 54:2164–2171, 2005

D iabetic nephropathy, responsible for >40% of end-stage renal disease (ESRD) in the U.S. (1), is the leading cause of end-stage renal failure in the western world. Approximately 25% of type 1 diabetic patients will develop overt diabetic nephropathy (2). While effective antihypertensive therapy, especially ACE inhibitors (3) and angiotensin II receptor blockers (4,5), can slow the progression of established disease, diabetic nephropathy continues to be a major public health problem (1). This is, to a large extent, because treatment begun after the development of overt clinical nephropathy does not arrest progression to ESRD (3–5). There is therefore a need to identify predictors of diabetic nephropathy risk early in the course of type 1 diabetes in order to develop effective prevention strategies.

Type 1 diabetes is commonly associated with a period of hyperfiltration (6–9) followed, in a subset of patients, by the development of persistent microalbuminuria (PMA) after as little as 7–10 years of type 1 diabetes (10–12). Microalbuminuria identifies type 1 diabetic patients who, on average, have more advanced diabetic nephropathy lesions compared with patients remaining normoalbuminuric (13–15). Moreover, PMA is associated with a 400–500% increase in the risk of progression to overt proteinuria and eventual ESRD (16,17).

Because of the relationship between AER and diabetic nephropathy, delineation of characteristics of normoalbuminuric patients that may predict subsequent albumin excretion rate (AER) patterns could be important. To that end, data from a collaborative international 5-year study designed to observe the natural history of early renal functional and structural changes in type 1 diabetes were analyzed (18,19). The objective of the present study was to develop associations between the baseline clinical and renal histologic characteristics of the participants with AER patterns that developed over a subsequent 5 years of clinical and laboratory observation.

RESEARCH DESIGN AND METHODS

Subjects for this Natural History Study (NHS) cohort were recruited by the International Diabetic Nephropathy Study Group consisting of three centers

(Montreal, Canada; Minneapolis, MN; and Paris, France), as previously described (18). Inclusion and exclusion criteria have been previously published.

The NHS cohort consisted of 243 patients with type 1 diabetes ranging in age from 10 to 40 years (16.8 ± 6.0 years; means ± SD) and with disease duration of 2–20 years (8.0 ± 4.2 years) (18). Eight patients with microalbuminuria at baseline, seven patients treated with renin-angiotensin system blockade, and one patient treated with an unknown antihypertensive drug during the 5 years of the study were excluded. Additional exclusion criteria are outlined below. All patients were normotensive, and no patient was on antihypertensive therapy at baseline.

Normal control subjects. Tissue was obtained from 87 normal nondiabetic kidney donors (aged 24 ± 8.6 years, range 8–47) at the time of kidney transplantation (19).

Study protocol. Patients were seen at baseline and scheduled to return every 3 months during the 5-year study (18). Each visit included measurement of blood pressure, HbA$_{1c}$ (A1C), and a timed overnight urine collection for AER determination using a sensitive fluorescence immunoassay (20). Blood pressure was measured by a Dinamap Vital Signs Monitor after 5 min in the seated position (18). Baseline blood pressure was defined as the mean of the first two visits in year 1 of the study. A1C was measured at each of the International Diabetic Nephropathy Study Group centers by high-performance liquid chromatography, as previously described (18). Baseline A1C was defined as the mean of the first two values in the 1st year of the study. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured annually, using iothalamate or inulin as clearance markers for GFR and para-amino hippurate for RPF, as previously detailed (18). Hyperfiltration was defined as GFR >130 ml^{-1} · min^{-1} · 1.73 m^{-2}. Percutaneous renal biopsies were obtained at baseline, as previously described (19). Light and electron microscopy measurements were performed at the University of Minnesota. Structural parameters included GBM width, fractional volumes of the mesangium [Vv (Mes/glom)], mesangial matrix [Vv (MM/glom)] and mesangial cells [Vv (MC/glom)], surface density of peripheral GBM [Sv (PGBM/glom)], glomerular volume (GV), total glomerular filtration surface (TFS), interstitial fractional volume [Vv (Int/cortex)], and index of arteriolar hyalinosis (IAH) (19). Normal values for these structural parameters have been previously published (19,21).

Definition of AER patterns and inclusion/exclusion criteria. A minimum of eight values over the 5 years of the study, with at least one of these values in year 5, was considered necessary to clearly establish an AER pattern and was an inclusion criterion. PMA was defined as two of three consecutive timed overnight urine AER values between 20 and 200 µg/min within a 6- to 12-month period (22). The following approaches were used to categorize patients into AER groups:

- Baseline AER values were calculated from the mean of the first two visits in year 1. If one of the values was discordant, i.e., one value was within the normoalbuminuric range (<20 µg/min) and one within the microalbuminuric range (20–200 µg/min), the value from a third visit was used to include or exclude the patient. The median was recorded if three values were used for the baseline AER value.
- If only two values were collected in the 1st year and they were discordant, the first collection in year 2 was used to define AER at baseline.
- If only one value was available in the 1st year, then the first collection in year 2 was used. If the values were concordant and in the normoalbuminuria range, the patient was included. If these values were discordant, the patient was excluded.
- If there were only two values obtained in year 5 of the study and they were discordant, the last value in year 4 was used.
- If only one value was obtained in year 5, the last value in year 4 was used. If these were discordant, the patient was excluded.

The 5-year AER excretion patterns for all of the subjects were blindly evaluated by two of the investigators (J.M.S. and M.M.), without knowledge as to their demographic, clinical, or renal structural data, and then classified into one of the four groups described below. Fifty-seven of 243 patients who had normoalbuminuria at baseline had insufficient follow-up AER data (i.e., less than eight values) to permit categorization into an AER pattern. A total of 170 subjects with sufficient AER data were available for analysis and were categorized into the four groups.

Figure 1 shows the representative patterns illustrating one example of a patient from each AER group. Group 1 (persistent normoalbuminuria [PNA], $n = 99$) had all follow-up AER values <20 µg/min (Fig. 1A). Group 2 (intermittent microalbuminuria [IMA], $n = 49$) had one or more AER levels >20 µg/min but did not meet criteria for PMA during the follow-up period (Fig. 1B). Group 3 (transient PMA [TPMA], $n = 14$) met criteria for PMA during follow-up but were normoalbuminuric at study exit (Fig. 1C). Group 4 (PMA, $n = 8$) were microalbuminuric at study exit (Fig. 1D).
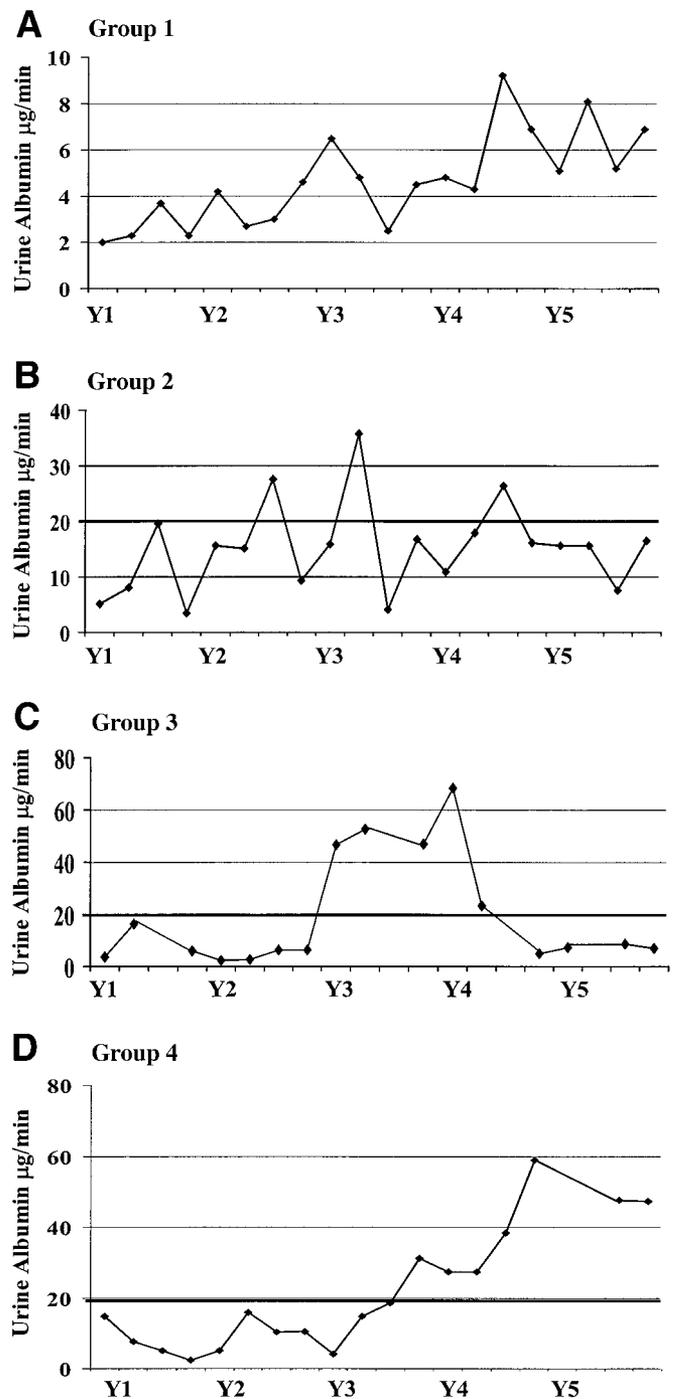
FIG. 1. Examples of the four AER patterns identified. Please note the varying y-axis scale for AER units in A–D. Albumin excretion was to be measured every 3 months. Years are denoted as Y1, Y2, Y3, Y4, and Y5. A: PNA pattern: a group 1 patient with all AER values <20 µg/min throughout the study. B: IMA pattern: a group 2 patient with occasional AER values > 20 µg/min (i.e., above the bold line) but with no two of three sequential values in the microalbuminuria range. C: TPMA pattern: a group 3 patient with sequential values >20 µg/min and a return to normoalbuminuria at study completion. D: PMA pattern: a group 4 patient meeting criteria for micoralbuminuria at the close of the study.

Statistical methods. Data are presented as means ± SD or, when skewed, median (range). Group 1 (PNA) was considered the reference group. Mean comparisons for each of the five groups to the reference group were assessed with one-factor ANOVA. The comparison of the type 1 diabetic cohort to the normal control subjects was done using t test. χ^2 analysis was used when comparing proportions. IAH was analyzed as a categorical variable with χ^2

TABLE 1
Baseline characteristics

	Group 1 (reference)*	Group 2 (IMA)†	Group 3 (TPMA)‡	Group 4 (PMA)§	P value (group 2)	P value (group 3)	P value (group 4)
n	99	49	14	8			
Age (years)	17.3 ± 6.4	15.3 ± 4.8	16.1 ± 6.3	17.0 ± 5.1	0.060	0.478	0.902
Sex (% male)	45%	37%	50%	75%	0.378	0.781	0.148
Age at onset (years)	8.7 ± 4.5	8.3 ± 4.1	8.5 ± 4.7	8.0 ± 3.1	0.615	0.852	0.682
Disease duration (years)	8.6 ± 4.7	7.0 ± 3.2	7.6 ± 3.9	9.0 ± 5.7	**0.038**	0.431	0.809
A1C (%)	8.4 ± 1.1	9.0 ± 1.2	8.7 ± 1.0	9.2 ± 1.3	**0.008**	0.356	0.064
Mean AER (μg/min)	4.1 ± 1.9	8.5 ± 6.0	9.9 ± 6.7	9.5 ± 8.6	**<0.001**	**<0.001**	**0.001**
Mean systolic blood pressure (mmHg)	114 ± 10	111 ± 9	112 ± 11	114 ± 11	0.148	0.649	0.936
Mean diastolic blood pressure (mmHg)	64 ± 6	62 ± 6	62 ± 7	64 ± 6	0.097	0.344	0.977
GFR $(ml \cdot min^{-1} \cdot 1.73 \ m^{-2})$	143 ± 28	136 ± 24	138 ± 23	163 ± 37	0.139	0.523	0.042
RPF $(ml \cdot min^{-1} \cdot 1.73 \ m^{-2})$	688 ± 177	645 ± 194	635 ± 198	615 ± 114	0.199	0.330	0.309
Filtration fraction	0.22 ± 0.04	0.23 ± 0.09	0.23 ± 0.07	0.25 ± 0.04	0.287	0.509	0.114

Data are means $\pm$ SD. Significant values are in bold. P values result from χ^2 analysis for proportion comparisons and one-factor ANOVA for all other variables between each individual group and the reference group. *n in group 1: GFR = 96, RPF and FF = 75. †n in group 2: RPF and FF = 46. ‡n in group 3: RPF and FF = 13. §n in group 4: RPF and FF = 7.

testing, that is, the proportion of subjects with IAH = 1 vs. IAH >1, rather than a continuous variable, as these data were not normally distributed. Subset analysis was also done on groups 3 and 4 combined in order to increase the statistical power for detection of association of predictor variables with abnormal AER patterns. All analyses were carried out using SAS PC version 8.2. P values <0.05 were considered statistically significant.

RESULTS

The median number of urine collections of these 170 subjects was 17 (range 8–21), with the lower quartile cutoff being 14 AER values. None of the subjects in groups 2, 3, or 4 had <10 urine values, whereas 4 of 99 patients in group 1 (reference group) had <10 values. There were no statistically significant differences between included ($n = 170$) and excluded ($n = 73$) patients for age, age at onset, or duration of type 1 diabetes (data not shown). However, baseline A1C levels were higher in the excluded patients versus the included patient cohorts (9.3 ± 1.5 vs. $8.7 \pm 1.2\%$, respectively, $P = 0.005$).

Baseline characteristics. There were no statistically significant differences for baseline age, sex, or age of diabetes onset between groups 2, 3, and 4 when compared with the reference group, i.e., group 1 (Table 1). Group 2 (IMA) patients tended to be younger (aged 15.3 ± 4.8 years, $P = 0.060$) and had statistically significantly shorter duration of type 1 diabetes (Table 1). A1C levels were higher in group 2 (IMA) than group 1 (PNA) (9.0 ± 1.2 vs. $8.4 \pm 1.1\%$, $P = 0.008$), with a similar trend found for group 4 (PMA) patients ($9.2 \pm 1.3\%$, $P = 0.064$) (Table 1). Mean systolic and diastolic blood pressure (mean of the values in year 1), RPF, and filtration fraction were not statistically significantly different for any of the groups (Table 1). When groups 3 and 4 were combined, there were no statistically significant differences for age, sex, age of onset, disease duration, A1C levels, blood pressure parameters, RPF, or filtration fraction compared with the reference group (data not shown).

All of the group means for baseline GFR values were in the hyperfiltration range ($>130 \ ml \cdot min^{-1} \cdot 1.73 \ m^{-2}$). The baseline GFR in group 4 (PMA) patients was significantly higher than in group 1 (PNA) patients (Table 1), but the GFR of groups 3 and 4 combined was $147 \pm 31 \ ml \cdot min^{-1} \cdot 1.73 \ m^{-2}$ and was not different from group 1 ($P = 0.509$). No statistically significant differences were observed in the proportion of patients with hyperfiltration (defined GFR $>130 \ ml \cdot min^{-1} \cdot 1.73 \ m^{-2}$) among the study groups: group 1, 61 of 99 (61%); group 2, 31 of 49 (63%); group 3, 8 of 14 (57%); and group 4, 7 of 8 (88%).

All patients, by design, were normoalbuminuric at baseline; however, some notable differences were found between the groups. Mean baseline AER (the mean of the first two or three AER values in year 1) was higher in groups 2, 3, and 4 compared with group 1 (Table 1). The mean baseline AER of groups 3 and 4 combined was significantly higher than the reference group (9.9 ± 7.2 μg/min, $P = <0.001$). All baseline AER values for group 1 (PNA) patients were <20 μg/min (data not shown). Eleven of 49 (22%) group 2 patients had one baseline AER value in the microalbuminuric range (median 26 μg/min [range 20–70]), 3 of 14 (21%) group 3 patients had one baseline AER value in the microalbuminuric range (median 47 μg/min [range 26–55]), and 1 of 8 (12%) group 4 patients had a single value in the microalbuminuric range (20.3 μg/min).

Renal structural studies. The main study cohort compared with the normal control subjects had increased GBM width, Vv (Mes/glom), and Vv (MM/glom) and decreased Vv (Int/cortex) (Table 2). This effect was retained even after age adjustment. No statistically significant differences were found for Vv (MC/glom), Sv (PGBM/glom), GV, or TFS between these two cohorts.

GBM width was greater in groups 3 (TPMA) and 4 (PMA) compared with the persistent normoalbuminuric reference group (Table 3). Sv (PGBM/glom) was greater in group 2 and TFS was greater in group 3 than group 1, but no statistically significant group differences were observed for GV (ANOVA P value = 0.353) (Table 3). Vv (Int/cortex) was greater in group 4 versus group 1 patients, while the other two groups did not differ from group 1 (Table 3). Group 1 was similar to the other groups for Vv (Mes/glom), Vv (MM/glom), and Vv (MC/glom) (Table 3). IAH was not significantly different among any of the groups.

Groups 3 and 4 combined had higher GBM width versus group 1 (476 ± 106 nm, $P = 0.001$). There was no longer a statistically significant difference in Sv (PGBM/glom) (0.14 ± 0.032, $P = 0.269$) or in Vv (Int/cortex) (0.10 ± 0.04, $P = 0.217$) for these comparisons; however, TFS was

TABLE 2
Comparison of normal control subjects with diabetic cohort

	Control subjects	Diabetic cohort	Crude comparison	Age-adjusted comparison
$n*$	87	178		
Age (years)	24.1 ± 8.6	16.6 ± 5.9	**<0.0001**	—
GBM width (nm)	342 ± 52	425 ± 76	**<0.0001**	**<0.0001**
Vv (Mes/glom)	0.200 ± 0.032	0.218 ± 0.044	**0.0001**	**<0.0001**
Vv (MM/glom)	0.085 ± 0.016	0.098 ± 0.029	**<0.0001**	**<0.0001**
Vv (MC/glom)	0.087 ± 0.022	0.090 ± 0.024	0.3563	0.2163
Sv (PGBM/glom)	0.131 ± 0.021	0.142 ± 0.023	**0.0001**	0.2453
GV (1×10^6 mm³)	1.67 ± 0.82	1.54 ± 0.54	0.4009	0.1861
TFS (1×10^3 mm²)	0.226 ± 0.123	0.218 ± 0.072	0.7317	0.6362
Vv (Int/cortex)	0.132 ± 0.038	0.092 ± 0.026	**<0.0001**	**0.0008**

Data are means ± SD. Significant values are in bold. *P* values result from *t* test between the normal control subjects and the diabetic cohort. *$n = 87$ for control subjects and 178 for diabetic cohort subjects, except for GV and TFS (control subjects, $n = 30$; diabetic cohort subjects, $n = 145$) and Vv (Int/cortex) (control subjects, $n = 26$; diabetic cohort subjects, $n = 151$).

greater in the combined groups 3 and 4 than the group 1 cohort ($0.25 ± 0.07$, $P = 0.033$). There were no differences in GV, Vv (Mes/glom), Vv (MM/glom), Vv (MC/glom), or IAH with groups 3 and 4 combined compared with group 1.

DISCUSSION

Progressive diabetic nephropathy structural injury develops over years to decades in silence before clinical and laboratory abnormalities such as albuminuria, hypertension, or declining GFR develop (13,14). The earliest clinical manifestations of diabetic nephropathy often represent already well-established renal injury (13,14,23,24). Microalbuminuria, currently considered the best clinical indicator of overt diabetic nephropathy risk, can be associated with diabetic nephropathy lesions that are so advanced as to overlap with those seen in patients with overt proteinuria and declining kidney function (13). Thus, waiting for clinical or laboratory manifestations of renal disease to emerge before initiating treatment may hinder efforts to prevent the ultimate progression of a substantial subset of patients to ESRD.

An important goal of this observational study, which focused on normoalbuminuric type 1 diabetic patients, was to observe emerging AER patterns and determine baseline predictors of those patterns. It is known that most normoalbuminuric patients will not have any AER in the microalbuminuric range over ≥5 years of repeated testing (25). The findings, however, that some type 1 diabetic patients have occasional values in the microalbuminuric range, but do not reach criteria for PMA, are also well described (25,26). The phenomenon of patients meeting these criteria for PMA and returning to normoalbuminuria on follow-up is now widely accepted and is discussed in greater detail below. Four albumin excretion patterns, considered to best fit the observed data, are described here: PNA, IMA, TPMA, and PMA. Clearly, this 5-year observation period, which begins in patients of varying age and diabetes duration, addressed only a circumscribed segment of the natural history of diabetic nephropathy. Thus, patients in any of the four groups in the present study could, over time, remain stable, convert to another group, or progress to overt proteinuria (16,17,27–30). The inclusion criterion of at least eight urine values over the 5-year period was established in order to reduce the possibility of misclassification. The study design also followed published guidelines for the diagnosis of microalbuminuria with multiple urine samples to increase the accuracy of classification (22).

Our study cohort may not be entirely representative of the type 1 diabetic patient population. While the original NHS cohort was not significantly different from patients at the study centers who chose not to participate (19), some

TABLE 3
Comparison of structural parameters

	Group 1 (reference)*	Group 2 (IMA)†	Group 3 (TPMA)‡	Group 4 (PMA)§	*P* value (group 2)	*P* value (group 3)	*P* value (group 4)
n	99	49	14	8			
GBM width (nm)	418 ± 67	411 ± 67	464 ± 113	497 ± 95	0.585	**0.030**	**0.004**
Vv (Mes/glom)	0.22 ± 0.05	0.21 ± 0.04	0.22 ± 0.04	0.21 ± 0.05	0.071	0.918	0.528
Vv (MM/glom)	0.10 ± 0.03	0.09 ± 0.02	0.10 ± 0.02	0.10 ± 0.04	0.199	0.844	0.653
Vv (MC/glom)	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.244	0.711	0.727
Sv (PGBM/glom)	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	0.13 ± 0.03	**0.013**	**0.024**	0.272
GV (1×10^6 mm³)	1.49 ± 0.43	1.53 ± 0.68	1.70 ± 0.72	1.76 ± 0.67	0.661	0.214	0.199
TFS (1×10^3 mm²)	0.21 ± 0.07	0.22 ± 0.08	0.25 ± 0.07	0.23 ± 0.07	0.182	**0.031**	0.398
Vv (Int/cortex)	0.09 ± 0.02	0.09 ± 0.02	0.09 ± 0.03	0.11 ± 0.06	0.898	0.904	**0.045**
IAH (%)	18 (19.8%)	7 (16.3%)	5 (35.7%)	2 (28.6%)	0.813	0.183	0.629

Data are means ± SD. Significant values are in bold. IAH represents the percent of patients with a score >1, and analysis was by χ^2. *P* values for all other variables result from one-factor ANOVA between each group and the reference group. *n in group 1: VvInt = 87, GV and TFS = 80, IAH = 91. †n in group 2: VvInt = 37, GV and TFS = 38, IAH = 43. ‡n in group 3: VvInt = 12, GV and TFS = 12. §n in group 4: VvInt, GV, and TFS = 7, IAH = 7.

selection bias cannot be entirely dismissed. Patients who were excluded because of inadequate follow-up AER data were more likely to have poor compliance and are presumably a population at increased risk, as suggested by their higher baseline A1C levels.

Sixty-four percent of the patients meeting criteria for PMA at some point during this 5-year study reverted to normoalbuminuria without treatment by the end of the study (Fig. 1C). This is similar to the 59% rate of spontaneous loss of PMA reported among 60 young type 1 diabetic patients who were followed for 16–18 years from adolescence to ~35 years of age and 25–35 years of diabetes duration (31). Fifty-eight percent (15 of 26) of microalbuminuric patients with a mean disease duration of 8.3 years (range 3–17) returned to normoalbuminuria in another study (32). Similarly, 40% of 220 type 1 diabetic microalbuminuric patients with diabetes duration >15 years were normoalbuminuric after 6 years of follow-up, and this was independent of ACE inhibitor treatment (27). Forsblom et al. (16) noted that 39% (7 of 20) of microalbuminuric type 1 diabetic patients with duration of disease >15 years were normoalbuminuric after 10 years of follow-up. Giorgino et al. (30), reviewing data from 352 microalbuminuric type 1 diabetic patients in the EURODIAB Prospective Complications Study followed for 7 years, found a 14% rate of progression to proteinuria, while 51% returned to normoalbuminuria. Recently, Hovind et al. (28) described an inception cohort of 277 type 1 diabetic patients followed for 1–21.5 years from disease onset. Seventy-nine patients (33%) developed microalbuminuria. During subsequent follow-up for a median of 7.5 years, 28 (35%) of these patients returned to normoalbuminuria (28). Fifteen patients who resumed to normoalbuminuria went on to develop microalbuminuria again. Thus, only 16% of the patients developing microalbuminuria were normoalbuminuric at the end of the study. It is not clear why the proportion of microalbuminuric patients who had reverted to normoalbuminuria at the end of this study is lower than in the studies described above (16,27,30,31). The differences do not simply appear to be related to study design or length of follow-up. One clear difference, however, is that the mean age of onset of type 1 diabetes was 27 years in the study by Hovind et al. versus the much more typical ages of 6–17 years in the present and other studies (27,30,31,33). From these observations, it can be hypothesized that microalbuminuria developing in adolescence or young adulthood is more likely to revert to normoalbuminuria than microalbuminuria developing in older adults or in patients with adult onset of type 1 diabetes.

Perkins et al. (27) reported that age, duration of microalbuminuria, A1C, systolic blood pressure, and lipid levels were independently associated with a ≥50% reduction in AER on follow-up. When the reversion to normoalbuminuria was analyzed dichotomously (as a binary variable), the outcome inferences were unchanged (B. Perkins, A. Krolewski, personal communication). The EURODIAB study also found lower baseline A1C and lipid levels as well as lower baseline AER values to be predictive of return to normoalbuminuria (30). The relatively small number of patients with microalbuminuria in the present report does not further clarify this issue, as no differences were observed in demographic, clinical, or renal structural or functional parameters between the subsets returning to normoalbuminuria (group 3) compared with those who were microalbuminuric (group 4) at the end of the study, although numerically higher GFR and GBM width was seen in the persistently microalbuminuric group.

All patients in the present study, by design, were normoalbuminuric at baseline. Baseline AER was lower in the persistently normoalbuminuric group (group 1) than the other three groups, while the latter were similar to one another. These data are consistent with earlier reports that higher AER values, even in the normoalbuminuric range, are predictors of progression to microalbuminuria or proteinuria (28,31). Dahlquist et al. (31) found that a single baseline AER >15 μg/min provided significant predictive value for the development of microalbuminuria or macroalbuminuria. In our study, all patients with one of their first two screening AERs >20 μg/min were ultimately classified in one of the groups with abnormal AER patterns. The baseline values, however, were unable to predict into which of these groups the patients would later be classified, and the published literature does not clarify this point. Clearly, patients with a single elevated AER are likely to have additional AER elevations and are at increased risk for diabetic nephropathy. However, the magnitude of this risk remains unclear (10,11,17,34).

This article reports that clinic blood pressures were not a predictor of subsequent AER patterns. However, in cross-sectional studies, Hansen et al. (35) found that higher ambulatory blood pressure values in normotensive type 1 diabetic patients were associated with higher AER values within the normoalbuminuric range. In addition, Torbjornsdotter et al. (36) reported that higher ambulatory blood pressure parameters were associated with worse diabetic nephropathy lesions in normoalbuminuric type 1 diabetic adolescent patients. Longitudinal studies by Lurbe et al. (37) found that nocturnal ambulatory blood pressure values and "dipper" status predicted the subsequent development of microalbuminuria in type 1 diabetic normoalbuminuric adolescents. Therefore, despite the efforts to standardize the clinic blood pressure measurement protocol, it may not provide a precise parameter estimate of the relationship between systolic blood pressure and diabetic nephropathy risk.

The association of hyperfiltration with risk of diabetic nephropathy is also controversial. Increased GFR has been found to be a predictor of diabetic nephropathy risk in some studies (6,12,38) but not in others (39–41). This lack of consensus may reflect variable definitions of progression and/or microalbuminuria (6,12), inclusion of newly diagnosed type 1 diabetic patients (39), variable definitions of hyperfiltration (6,38), and unusual datasets such as marked hyperfiltration among microalbuminuric patients (12). The inclusion of patients under very poor glycemic control (38) may also make it difficult to interpret the importance of GFR with certainty, as there is a known association between glycemia and GFR (9,42). The mean GFR values in each of the groups in the present study were >130 ml · min^{-1} · 1.73 m^{-2}, and the frequency of hyperfiltration was not different among them. GFR and GBM width were both higher in the patients with PMA (group 4) versus the persistent normoalbuminuric group (group 1),

suggesting an association with diabetic nephropathy risk. This difference remained when these GFR data were adjusted for A1C ($P = 0.048$). However, GBM width was also increased in the subjects who were transiently microalbuminuric (group 3), despite having similar GFR values to the reference group (group 1). Combining data from groups 3 and 4, the GFR difference was no longer evident, while the findings of greater GBM width persisted. Thus, as previously reported for our entire NHS cohort (19), there was no clear cross-sectional association between hyperfiltration and diabetic nephropathy lesions nor can one conclude from these reports that hyperfiltration is a risk factor for progression. Only well-designed long-term natural history or treatment studies will be able to determine whether increased GFR is an independent risk factor for diabetic nephropathy.

As previously reported for the total NHS cohort (19), our type 1 diabetic subjects had diabetic nephropathy lesions despite normal blood pressure and normoalbuminuria. The increases in GBM width in groups 3 and 4 are of concern, because young microalbuminuric type 1 diabetic patients with greater GBM width are more likely to progress to overt proteinuria (43). Longer-term follow-up of larger numbers of patients is necessary to clarify the prognostic relevance of these findings. Previous NHS data suggest that GBM width tends to increase linearly with increasing type 1 diabetes duration, while Vv (Mes/glom) changes little in the first 15 years and increases more rapidly thereafter (44). This may explain the association between greater GBM width at earlier stages of the disease and the later development of microalbuminuria, whereas Vv (Mes/glom) is the stronger correlate of albuminuria and declining GFR with longer type 1 diabetes duration (13,45).

The decision to exclude patients using ACE inhibitors or angiotensin II receptor blockers during the study was based on studies showing that use of these medications reduced AER in type 1 diabetic patients (46,47), making subsequent AER patterns difficult to interpret. The removal of these patients from the groups selected for AER patterns had little impact on the study findings other than increasing the significance of GBM width.

All of the groups had mean A1C levels that are above the American Diabetes Association–recommended level of 7% (48). Patients with IMA (group 2) had higher A1C levels and shorter disease duration than PNA patients (group 1). Since hyperglycemia is an important risk factor for diabetic nephropathy, patients in this group may be at greater risk with longer type 1 diabetes duration (26,49). Gorman et al. (33) found a direct relationship between baseline A1C and risk of microalbuminuria in 76 type 1 diabetic adolescents. Bojestig et al. (32), in a 10-year follow-up study, reported that patients returning to normoalbuminuria from microalbuminuria had lower A1C at the time of detection of microalbuminuria than those that remained microalbuminuric (6.7 ± 0.2 vs. $7.7 \pm 0.4\%$, $P = 0.03$).

This study examined demographic, clinical, and renal structural and functional variables as predictors of AER patterns. However, the measurement of other potential biomarkers, such as urinary podocytes or the loss of podocyte-specific proteins, inflammatory markers, such as C-reactive protein or interleukin-6, etc., that have been associated with the risk of diabetic nephropathy could provide additional opportunities for further work in this cohort (50,51).

Current American Diabetes Association guidelines recommend treatment with ACE inhibitors in all type 1 diabetic patients developing PMA (48,52). The present study and others, as discussed above, found a high rate of spontaneous return to normoalbuminuria, especially in younger patients (16,27,30–32). The ultimate course of patients that revert to normoalbuminuria, in particular, is not yet defined. Current treatment recommendations, however, could obscure the natural history of this disorder. These recommendations, on the one hand, could result in unnecessary treatment of patients at low risk of progression and, on the other hand, may mask progression toward overt proteinuria (47). Perhaps one option would be to discontinue ACE inhibitor therapy for a 2-month period every few years in normotensive type 1 diabetic patients and decide about therapy reinitiation based on repeated AER values while off these medications. Perhaps these current guidelines should be reevaluated in the light of the more recent information. Finally, it is clear that much remains to be learned about the early natural history of diabetic nephropathy in type 1 diabetic patients.

APPENDIX

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