PC-1 Amino Acid Variant Q121 Is Associated With a Lower Glomerular Filtration Rate in Type 2 Diabetic Patients With Abnormal Albumin Excretion Rates

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OBJECTIVE — To study the relationships between the PC-1 K121Q variant and diabetic nephropathy (DN) in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A total of 125 patients with type 2 diabetes and abnormal albumin excretion rate (AER) (range 20–5,416 μg/min) were followed up for 4 years with repeated measurements of glomerular filtration rate (GFR). Genomic DNA was extracted from all patients, and the PC-1 K121Q polymorphism was determined by the PCR *Ava*II restriction enzyme. A subset of 64 patients underwent a percutaneous kidney biopsy at baseline, and glomerular structure was analyzed by electron microscopic morphometric analysis. At baseline, age (56 ± 8 vs. 59 ± 7 years), BMI (28.3 ± 4.3 vs. 28.6 ± 3.7 kg/m^2), known duration of type 2 diabetes (11.1 ± 7 vs. 11.9 ± 8 years), and HbA$_{1c}$ (8.6 ± 1.8 vs. $8.4 \pm 1.7\%$) were similar in K121K (KK, $n = 87$, 73 men/14 women) and XQ (35 K121Q + 3 Q121Q, $n = 38$, 27 men/11 women) patients. Baseline GFR was 96 ± 28 ml · min^{-1} · 1.73 m^{-2} and was related ($P = 0.01$–0.001) to age, known diabetes duration, and systolic blood pressure.

RESULTS — XQ patients had lower GFR ($P < 0.05$) than KK patients (88 ± 30 vs. 100 ± 26 ml · min^{-1} · 1.73 m^{-2}); this difference persisted also after factoring in age and known diabetes duration. The rate of progression of DN was similar in KK and XQ patients: %ΔGFR was 4.1/year (median, range: 22.9–30.6) vs. 4.2/year (9.8–26.7). Morphometric parameters of diabetic glomerulopathy were similar in the two genotype groups.

CONCLUSIONS — Among patients with type 2 diabetes with abnormal AER, those carrying the Q PC-1 genotype have more severe DN but not a faster GFR decline than KK patients, thus suggesting faster DN development since diabetes diagnosis in XQ patients.

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Diabetic nephropathy (DN) is a complex disease with environmental and genetic (1) backgrounds contributing to its development and progression. Although environmental determinants (including, among others, metabolic control [2], blood pressure [3], and smoke [4]), are quite well established, genetic factors are mostly unknown (5).

Insulin resistance has been suggested to play a role in DN in both type 1 diabetic (6) and type 2 diabetic (7) patients. Insulin resistance also has a genetic background (8); therefore, it is possible that insulin resistance and DN share some common genetic determinants. In line with this hypothesis, it has recently been reported that a plasma membrane glycoprotein PC-1 amino acid variant (Q121), which associates with insulin resistance in several populations (9,10), also associates with DN progression in some (11), although not all (12), European populations of type 1 diabetic patients with albuminuria. The Q121 PC-1 variant has also been reported to be associated with higher risk of early-onset end-stage renal failure in type 1 diabetic patients from the U.S. (13). Whether the Q121 PC-1 variant is associated with features of DN also in type 2 diabetic patients is unknown.

The present study was aimed at investigating the relationship between the PC-1 gene and both severity and progression of kidney disease in a cohort of type 2 diabetic patients with abnormal albumin excretion rate (AER). In addition, in a subset of patients, the impact of the PC-1 gene on glomerular structure was investigated.

RESEARCH DESIGN AND METHODS

Patients

This study was designed to explore the associations between the polymorphisms of candidate genes and progression of

DN. Inclusion criteria were as follows: type 2 diabetes diagnosed according to World Health Organization criteria (1985), ≤70 years of age, serum creatinine <2.4 mg/dl, persistent microalbuminuria (AER ≥20 to <200 μg/min) or macroalbuminuria (AER ≥200 μg/min) in at least two of three 24-h urine collections, and absence of nondiabetic renal disease. Caucasian type 2 diabetic patients living in northeast Italy were recruited by two diabetic units (Unit A and Unit B) at the University of Padua. Only data of patients with a follow-up of at least 2 years and at least three glomerular filtration rate (GFR) measurements were analyzed (total of 125 patients).

These studies have been approved by the local Ethical Committee of the University of Padova and the National Health Ministry, and each patient gave written informed consent before entry into the study.

Unit A. All patients recruited had a kidney biopsy performed at baseline as part of an ongoing study on renal structural-functional relationships in type 2 diabetes. All of them did not have renal biopsy contraindication, including single kidney, serious stone disease, anticoagulant therapy, and severe and uncontrolled hypertension. In no case were renal biopsies performed for clinically indicated diagnostic purposes. A total of 72 consecutive Caucasian patients met the inclusion criteria and agreed to participate. In four patients, renal tissue was not adequate for electron microscopic morphometric analysis, two patients died before 2 years of follow-up (one from myocardial infarction and one from stroke) and were then excluded, and two dropped out of the study. Thus, a total of 64 type 2 diabetic patients were included in the present study. In all patients, blood samples for DNA extraction were taken at the baseline observation, when the kidney biopsy was performed.

Unit B. Inclusion criteria for patients enrolled by Unit B were identical to those of Unit A, with the exception of AER levels, which had to be ≥200 μg/min (i.e., macroalbuminuria). Moreover, patients recruited by this unit had blood samples for DNA extraction taken during the first 2 years of follow-up. Of the 63 patients identified and willing to participate in the study, 2 died because of myocardial infarction before 2 years of follow-up. Thus, a total of 61 type 2 diabetic patients were included in the present study from Unit B. A total of 46 patients with microalbuminuria and 79 with proteinuria were included.

Overall, 116 patients were on either ACE inhibitors or angiotensin II receptor 1 blockers; 9 patients had side effects from these agents and received dihydropyridinic calcium antagonists, diuretics, and other antihypertensive drugs. Among patients treated with ACE inhibitors or angiotensin II receptor 1 blockers, dihydropyridinic calcium antagonists were added in 55 (44%) patients, whereas diuretics and α- or β-blockers were added in 54 (43%) patients.

Time elapsed since diabetes diagnosis (i.e., known diabetes duration) was calculated from the calendar year of data collection minus the calendar year of diabetes diagnosis.

Follow-up

Data presented are from 125 patients, who were followed up for at least 2 years and had at least three GFR measurements. Data from five patients (Unit A) who died during follow-up (two from cancer, one from end-stage renal disease, and two from myocardial infarction) have been analyzed because these events developed after >2 years of follow-up. Median duration of follow-up was 4 years (2–10). Measurements of HbA_{1c} (14), overnight fasting serum creatinine (15), AER, and GFR were carried out at baseline and every 6 months. At baseline and during the follow-up, blood pressure was regularly measured, and treatment was confirmed or changed every 2 months according to current guidelines. Cigarette smoking history was assessed by a questionnaire.

Measurements

AER was measured by the immunoturbidimetric technique (16) in sterile urine specimens. AER was defined by the geometric mean of three AER overnight measurements evaluated 3–5 days after washout from antihypertensive treatment. GFR was determined by a modeling analysis of plasma decay of ^{51}Cr-labeled ethylenediaminetetra-acetate from frequent blood samples over 300 min after abrupt injection of tracer, as described elsewhere. The normal values in a group of age-matched normal control subjects were 85–135 ml · min^{-1} · 1.73 m^{-2} (17).

Renal structure

Kidney biopsies were performed in patients from Unit A ($n = 64$) under ultrasound guidance by an experienced investigator (P.F.). After kidney biopsy, tissue was immediately examined under a dissecting microscope to ensure adequate numbers of glomeruli and processed for light, electron, and immunofluorescence microscopy. Electron microscopic morphometric analysis was performed on three open glomeruli per biopsy. Glomeruli were photographed with a Hitachi H600 electron microscope at ×3,900 to obtain photomontages of the entire glomerular profile to estimate mesangial fractional volume [Vv(mes/glom)], the fraction of the glomerulus occupied by mesangium, as previously described (18). Normal values are 0.19 ± 0.03. Another set of micrographs, photographed at ×12,000 by entering the glomerulus at its lowest segment and systematically sampling about 20% of the glomerular profile, was used to estimate glomerular basement membrane width (19). Normal values are 310 ± 38 nm. The same micrographs were used to estimate the fraction of the glomerulus occupied by mesangial cells [Vv(MC/glom)] and matrix [Vv(MM/glom)]. Normal values for both are 0.09 ± 0.03. Normal ranges were obtained from a group of 27 normal kidney donors (14 men/13 women) matched for age (56 ± 10 years) with the patients studied.

Genotyping

High–molecular weight DNA for genotyping was extracted from peripheral blood (5–10 ml), which was taken into EDTA-containing tubes, frozen whole, and stored at −30°C until extraction. Genomic DNA was extracted by the proteinase K–phenol/chloroform standard method; resuspended in 10 mmol/l Tris-HCl, pH 8.0, 1 mmol/l EDTA; and stored at 4°C. PCR technique, specific primers, and experimental conditions used for genotyping with the *Ava*II restriction enzyme have been previously described (9).

Statistical analysis

Data are reported as means ± SD or median (range). Values of AER, not normally distributed, were logarithmically transformed before analysis and are expressed as median and range.

Mean differences were compared by unpaired Student's *t* or Mann-Whitney *U*

Table 1—*Clinical features of type 2 diabetic patients divided on the basis of their genotype*

	All patients	KK	XQ
n	125	87	38
Sex (M/F)	100/25	73/14	27/11
Age (years)	57 ± 8	56 ± 8	59 ± 7
BMI (kg/m^2)	28.4 ± 4	28.3 ± 4.3	28.6 ± 3.7
Known diabetes duration (years)	11.4 ± 7	11.1 ± 7	11.9 ± 8
GFR (ml · min^{-1} · 1.73 m^{-2})	96 ± 28	100 ± 26	88 ± 30*
Serum creatinine (μmol/l)	97 ± 27	96 ± 26	99 ± 28
AER (μg/min)	223 (20–5,416)	230 (20–5,416)	205 (20–1,528)
sBP at baseline (mmHg)	152 ± 19	152 ± 18	152 ± 20
dBP at baseline (mmHg)	88 ± 10	88 ± 10	86 ± 10
sBP at follow-up (mmHg)	154 ± 20	155 ± 20	152 ± 18
dBP at follow-up (mmHg)	88 ± 9	88 ± 10	86 ± 8
HbA$_{1c}$ (%)	8.5 ± 1.7	8.6 ± 1.8	8.4 ± 1.7
Triglycerides (mmol/l)	2.3 ± 2.4	2.2 ± 1.2	2.6 ± 1.9
Total cholesterol (mmol/l)	5.6 ± 1.2	5.5 ± 1.2	5.9 ± 1.3
Smokers (%)	50	46	56
Lipid-lowering therapy (%)	27	26	29

Data are means ± SD or median (range) unless otherwise indicated. *$P < 0.05$. dBP, diastolic blood pressure; sBP, systolic blood pressure.

tests as appropriate. Genotype distribution in different groups was compared by χ^2 analysis.

Linear regression analysis on all GFR values of each patient during the follow-up period was applied to estimate the absolute individual rate of GFR decline and then expressed as %ΔGFR/year change from baseline. Linear regression analysis could also be applied in the 10 patients (i.e., 8% of the entire cohort studied) who were hyperfiltering at baseline (GFR >135 ml · min^{-1} · 1.73 m^{-2}) given that their rate of GFR change was constant over the time.

Univariate and multivariate analyses were used to correlate independent variables, with the dependent variable being either baseline GFR or GFR decline. For this analysis, data not normally distributed were logarithmically transformed.

Based on the role of the PC-1 K121Q polymorphism in modulating the rate of DN progression in type 1 diabetic patients, our sample size has a power of 98% to detect a difference of the same magnitude of that previously reported (11). A *P* value <0.05 was considered significant.

RESULTS — The clinical features of patients studied are shown in Table 1. A total of 87 patients (70%) carried the K121K genotype (KK patients), 35 (28%) the K121Q genotype, and 3 (2%) the Q121Q genotype; data for patients from the latter two groups, who were named XQ patients, were pooled and analyzed together. The Q allele frequency was 0.16, which is similar to that reported in several European populations (9,10), and it was in Hardy-Weinberg equilibrium.

No difference in the Q allele frequency was observed between 46 patients with microalbuminuria (0.18) or 79 patients with macroalbuminuria (0.15).

KK and XQ patients were similar in terms of sex distribution, age, known diabetes duration, AER, serum creatinine, systolic and diastolic blood pressure at baseline and at follow-up, HbA$_{1c}$, lipid profile, lipid-lowering therapy (statins or fibrates), and smoking status (i.e., patients being defined as current smokers or not) (Table 1). Also antidiabetic and antihypertensive treatments were not different across the two genotype groups (data not shown).

Baseline GFR was 96 ± 28 ml · min^{-1} · 1.73 m^{-2} (range 21–160 ml · min^{-1} · 1.73 m^{-2}) and in the univariate regression analysis, significantly correlated with age ($P < 0.01$), known diabetes duration ($P < 0.01$), and systolic blood pressure at baseline ($P = 0.001$) but not with HbA$_{1c}$ ($P = 0.6$), triglycerides, cholesterol, smoking status, and sex. XQ patients had a significantly lower baseline GFR (88 ± 30 vs. 100 ± 26 ml · min^{-1} · 1.73 m^{-2}, $P = 0.032$; Table 1). The difference in baseline GFR across the two genotype groups remained significant when adjusted for either age and strongly related known diabetes duration ($P = 0.047$), or sex ($P = 0.03$), or baseline systolic blood pressure ($P = 0.04$), or HbA$_{1c}$ ($P = 0.03$), or triglycerides ($P = 0.02$), or total cholesterol ($P = 0.02$). When all these variables were considered together, the difference in baseline GFR between the two genotype groups was still significant ($P = 0.049$).

During follow-up (median duration, 4.0 years; range, 2–10 years), median kidney function decline (i.e., %ΔGFR/year) was 4.2 (range, 22.9–30.6). In univariate regression analysis, a significant correlation was observed between the rate of GFR decline and systolic blood pressure at baseline ($P = 0.001$) and at follow-up ($P = 0.015$) and AER ($P < 0.001$) but not with time of known diabetes duration, HbA$_{1c}$, triglycerides, cholesterol, and smoking status. The rate of GFR decline was not different between KK and XQ patients: 4.1%/year (median, range: 22.9–30.6) versus 4.2 (9.8–26.7), respectively (NS).

Similarly, glomerular structure measurements were not different between 40 KK and 24 XQ patients who were representative of the entire cohort as far as clinical features are concerned (data not shown). In detail, glomerular basement membrane width was 449.5 ± 90 vs. 465.3 ± 132 nm, Vv(mes/glom) was 0.278 ± 0.08 vs. 0.270 ± 0.07, and Vv(MM/glom) was 0.14 ± 0.05 vs. 0.13 ± 0.04 in XQ and KK patients, respectively (NS for all).

CONCLUSIONS — Our data indicate that, among type 2 diabetic patients with abnormal AER, those carrying the Q121 PC-1 amino acid variant (9–10,20,21), although having similar diabetes duration, have lower baseline GFR. At variance, the rate of subsequent GFR decline was not different in KK versus XQ patients. These data suggest a faster DN development since diabetes diagnosis in XQ patients.

The biology of this association is unknown. It might be based on insulin resistance and/or compensatory hyperinsulinemia, which have been associated with reduced GFR in both type 1 diabetes (22) and nondiabetic glomerular diseases (23). Hyperinsulinemia may, in fact, stimulate renal sodium reabsorption, leading to volume expansion, increased

adrenergic activity, and hypertension (24,25). Indeed, systolic blood pressure was correlated with baseline GFR in our cohort; in addition, among untreated type 2 diabetic patients, those carrying the XQ genotype have higher systolic blood pressure (10). Thus, although in our present series differences in systolic blood pressure were not detectable, very likely because of the ongoing antihypertensive treatment, one could speculate that the lower GFR in XQ patients is the consequence of different blood pressure levels either too subtle to be detected without 24-h blood pressure monitoring or occurring before treatment was started. Also, other surrogates of insulin resistance were not different between the two genotype groups. The reasons for this lack of association probably reside in the metabolic background of the patients studied. Because of DN and related abnormalities (i.e., hypertension and dyslipidemia), these patients show an acquired "insulin resistance phenotype" that may be indistinguishable from the phenotype given by the intrinsic genetically determined insulin resistance. Lack of association between the K121Q polymorphism and insulin resistance among patients with DN has also been previously reported in type 1 diabetic patients (11,13), thus indicating that this may be a general phenomenon.

An important determinant of GFR is the degree of diabetic glomerulopathy, especially mesangial expansion (18,26); GFR was indeed inversely related to mesangial volume fraction ($r = 0.4$, $P = 0.001$) in our present series. However, because the morphometric glomerular parameters were similar in the two genotype groups, the degree of diabetic microangiopathy is unlikely to account for the differences in baseline GFR. As compared with KK individuals, XQ patients might have an impaired hemodynamic adaptation to glomerular injury or severe tubulo-interstitial and vascular lesions (macroangiopathy), as previously described in a subset of type 2 diabetic patients (19). This possibility is supported by the association between the PC-1 Q121 variant and macroangiopathy at the coronary artery district (27,28).

At variance with data here presented in type 2 diabetic patients, it has been reported that, in European type 1 diabetic patients, the Q121 PC-1 variant is a risk factor for faster DN progression (11) but not development (29,30). The association

between the Q121 variant and faster DN progression in type 1 diabetes has not been confirmed among the Danish population (12); however, this is the same population where no association between the Q121 variant and insulin resistance was detectable (31), thus suggesting that these negative results may be specific for Danes. Very recently, it was reported that, in type 1 diabetic patients from the U.S., the Q121 PC-1 variant is a risk factor for early-onset end-stage renal failure (13). This might be the consequence of faster DN progression in XQ patients, thus resembling our previous findings in type 1 diabetic patients (11). However, it may also be the consequence of faster DN development since diabetes diagnosis in XQ patients, a possibility that would be in line with our present data in type 2 diabetic patients. It may be that different genetic determinants may differently influence several aspects and stages of DN in patients with different pathophysiology backgrounds (5) and may also interact differently with ongoing antihypertensive treatments.

Overall, previous (11,13) and present data suggest that, in both type 1 diabetic and type 2 diabetic patients, the PC-1 gene is involved in some aspects of DN.

Although the cohort enrolled by Unit B could have been affected by a mortality bias during the first 2 years of follow-up (i.e., before the blood sample for DNA extraction had been withdrawn), this risk is likely to have minimal, if any, impact on the positive genotype/phenotype association described here because the mortality rate was 3% per year. Thus, during the first 2-year follow-up, only 3 of 50 patients from Unit B may have died. In addition, and more importantly, because the PC-1 Q121 variant is associated with several cardiovascular risk factors (27,28), a mortality bias, if any, should have primarily affected Q-carrying individuals, weakening the association between the Q121 variant and reduced GFR.

Finally, given the small sample size, our degree of statistical significance cannot exclude the risk of a false-positive result. Confirmative studies in a larger sample size and different populations are therefore needed.

In conclusion, our data indicate that, among type 2 diabetic patients with nephropathy, those carrying the Q121 PC-1 variant have a lower baseline GFR but not a higher GFR decline, thus suggesting

they may suffer from a faster development of DN since diabetes diagnosis. Only long-term, possibly larger, longitudinal studies will clarify the nature of this association.

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