

## Brief Genetics Report

# A PC-1 Amino Acid Variant (K121Q) Is Associated With Faster Progression of Renal Disease in Patients With Type 1 Diabetes and Albuminuria

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Insulin resistance characterizes type 1 diabetes in patients with albuminuria. A PC-1 glycoprotein amino acid variant, K121Q, is associated with insulin resistance. We examined the impact of the PC-1 K121Q variant on the rate of decline of the glomerular filtration rate (GFR) by creatinine clearance derived from the Cockcroft-Gault formula in 77 type 1 diabetic patients with albuminuria who were followed for an average of 6.5 years (range 2.5–15). Patients carrying the Q allele ( $n = 22$ ; 20 with KQ and 2 with QQ genotypes) had a faster GFR decline than those patients with the KK genotype ( $n = 55$ ) (median 7.2 vs. 3.7 ml · min<sup>-1</sup> · year<sup>-1</sup>; range 0.16 to 16.6 vs. -3.8 to 16.0 ml · min<sup>-1</sup> · year<sup>-1</sup>;  $P < 0.001$ ). Significantly more patients carrying the Q allele belonged to the highest tertile of GFR decline (odds ratio = 5.7, 95% CI 4.1–7.2,  $P = 0.02$ ). Levels of blood pressure, HbA<sub>1c</sub>, and albuminuria were comparable in the two genotype groups. Albuminuria ( $P = 0.001$ ), mean blood pressure ( $P = 0.046$ ), and PC-1 genotype ( $P = 0.036$ ) independently correlated with GFR decline. Because all patients were receiving antihypertensive treatment, the faster GFR decline in the patients carrying the Q allele could be the result of reduced sensitivity to the renoprotective effect of antihypertensive therapy. PC-1 genotyping identifies type 1 diabetic patients with a faster progression of diabetic nephropathy. *Diabetes* 49:521–524, 2000

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AER, albumin excretion rate; ANOVA, analysis of variance; DN, diabetic nephropathy; GFR, glomerular filtration rate; MAP, mean arterial pressure.

**T**he rate of progression of diabetic nephropathy (DN) varies greatly among individuals. Differences in environmental factors may explain part of this variability, but genetic factors, which are mostly unknown, also play an important role (1–6).

Insulin resistance characterizes type 1 diabetes in patients with albuminuria and their nondiabetic first-degree relatives (7,8) and underlies many of the alterations of DN, including high blood pressure, lipid abnormalities, increased left ventricular mass, and a family history of hypertension and cardiovascular disease (9). These observations suggest that insulin resistance is likely to precede and play a role in the vascular damage of DN. As for DN, insulin resistance also recognizes genetic determinants (10). It is possible, therefore, that DN and/or its progression and insulin resistance share a common genetic background.

The molecular mechanisms of insulin resistance are not completely understood (10,11). Recently, the role of specific inhibitors of insulin signaling has been described in human insulin resistance (10,11). These inhibitors include membrane glycoprotein PC-1 (12–16). We have recently reported an amino acid polymorphic variant of PC-1 glycoprotein, K121Q, which is strongly associated with insulin resistance (17). Because of the relationship between insulin resistance and DN, we have examined the impact of the PC-1 Q121 amino acid variant on the rate of DN progression.

From January 1995 to April 1997, 77 type 1 diabetic patients with albuminuria attended the diabetes units of five participating centers (as described in RESEARCHDESIGNANDMETHODS) and were studied. Information was available over an average retrospective follow-up of 6.5 years (range 2.5–15). As a selection criterion, patients had to have persistent proteinuria at baseline, but 20% of the patients had an albumin excretion rate (AER) <300 mg/24 h at the time of genotyping. This development was likely to be the result of the antihypertensive therapy that all of the patients received at least 2 years before baseline observation. At the time of genotyping, 95% of the patients were receiving ACE inhibitors alone (10%) or in combination with diuretics (45%) or calcium channel blockers and diuretics (40%). The remaining 5% of the patients were treated with calcium-channel blockers, diuretics, and  $\alpha$ -blockers. No major change in antihypertensive therapy took place

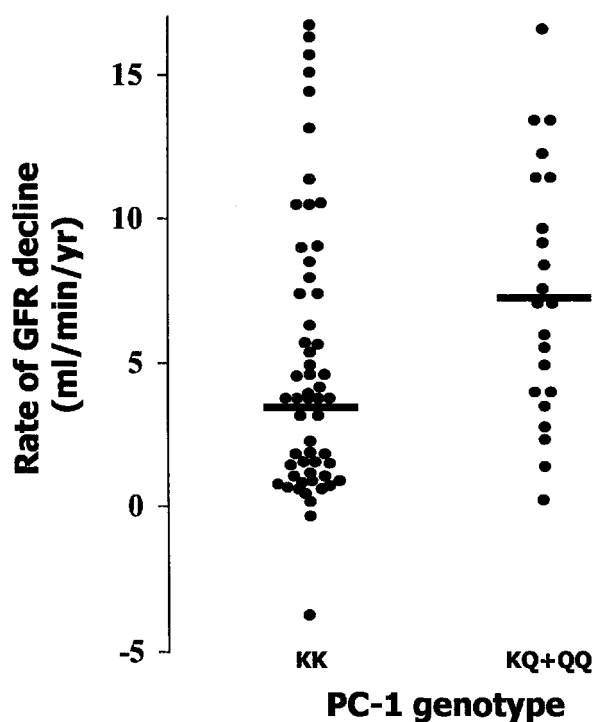


FIG. 1. Rate of GFR decline in 77 type 1 diabetic patients with albuminuria according to PC-1 K121Q genotype. —, Median values.

for at least 4 years before the time of genotyping. The mean glomerular filtration rate (GFR), which was calculated by the Cockcroft-Gault formula (18), was  $96 \pm 3 \text{ ml} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$  at baseline. Of the patients studied, 55 (71%) had the KK genotype, 20 (26%) had the KQ genotype, and 2 (3%) had the QQ genotype. Patients with the KQ and QQ genotypes were considered together and named the Q-carrying patients. The Q-allele frequency was 16%, which is similar to that reported in the general population (17), and was in Hardy-Weinberg equilibrium. No significant difference was observed in the Q-allele frequency between Italian and British patients (15.7 and 16.0%, respectively).

In the total cohort, the median rate of GFR decline was  $4.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ . No heterogeneity in GFR decline was found among the participating centers (data not shown). Figure 1 shows that the rate of GFR decline was higher in Q-carrying patients than in KK patients (median  $7.2$  vs.  $3.7 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ , range  $0.16$  to  $16.6$  vs.  $-3.8$  to  $16.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ ,  $P < 0.001$  by two-way analysis of variance [ANOVA]). Thus, patients were subdivided in tertiles of GFR decline:  $0.97$  ( $-3.8$  to  $2.3$ ),  $4.4$  ( $2.8$  to  $7.2$ ), and  $11.0$  ( $7.6$  to  $16.6$ )  $\text{ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ . The Q-allele frequencies were 4, 23, and 19% in tertiles 1, 2, and 3, respectively, with Q carriers having a higher risk to be fast (tertile 3) rather than slow (tertile 1) progressors (odds ratio = 5.7, 95% CI 4.1–7.2,  $P = 0.02$ ). The two PC-1 genotype groups were otherwise comparable for clinical variables (Table 1), and no clear difference in the insulin-resistant phenotype between KK and Q carriers was observed. The reasons for this lack of association are likely to reside in the metabolic and genetic background of the patients studied. Because of hyperglycemia, lipid abnormalities, hypertension, and impaired renal function, type 1 diabetic patients with nephropathy show an acquired insulin-resistant phenotype

TABLE 1

Clinical features of 77 type 1 diabetic patients with albuminuria according to PC-1 K121Q genotype

	KK	KQ + QQ
Age (years)	$42.3 \pm 1.2$	$41.7 \pm 2.1$
Sex (M/F)	36/19	12/10
Duration of diabetes (years)	$26.2 \pm 1.2$	$25 \pm 1.9$
BMI ( $\text{kg}/\text{m}^2$ )	$24.7 \pm 0.4$	$24.4 \pm 0.5$
Total cholesterol (mmol/l)	$5.8 \pm 0.2$	$5.6 \pm 0.2$
HDL cholesterol (mmol/l)	$1.4 \pm 0.1$	$1.4 \pm 0.1$
Triglycerides (mmol/l)	$1.7 \pm 0.2$	$1.4 \pm 0.1$
HbA <sub>1c</sub> (%)	$8.5 \pm 0.2$	$8.9 \pm 0.4$
MAP (mmHg)*	$99 \pm 1.3$	$100 \pm 1.7$
AER (mg/day)	460 (50–4,800)	480 (90–1,300)
Duration of follow-up (years)	7.2 (2.5–15.0)	6.0 (2.5–15.0)

Data are means  $\pm$  SE or median (range). \*ACE inhibitors were used for an average of  $5 \pm 4$  years in KK vs.  $4 \pm 3$  years in Q-carrying patients (NS).

that is difficult to distinguish from the phenotype determined by the intrinsic genetically determined insulin resistance. Moreover, because insulin resistance has a genetically heterogeneous background, type 1 diabetic patients with nephropathy are likely to carry several genetic determinants, all of which, eventually, would end up in a similar indistinguishable insulin-resistant phenotype. However, unlike PC-1, other genetic determinants of insulin resistance may not necessarily play a role in the rate of GFR decline.

In univariate analysis, individual GFR decline correlated with mean arterial pressure (MAP) ( $r = 0.24$ ,  $P = 0.04$ ) and AER ( $r = 0.48$ ,  $P = 0.0003$ ) but not levels of HbA<sub>1c</sub> at time of genotyping. The lack of correlation between HbA<sub>1c</sub> levels and GFR decline in this study, as in a previous study (19), may be due to the overriding effect of blood pressure. In a multiple regression analysis, the PC-1 genotype ( $P = 0.036$ ), AER ( $P = 0.001$ ), and MAP ( $P = 0.046$ ) were independent determinants of GFR decline, and together they accounted for 35% of the GFR loss, 11% of which is attributed to the PC-1 genotype. Thus, the Q121 PC-1 amino acid variant is associated with faster DN progression, independently of important environmental progression promoters. Our present findings are consistent with and reinforce the evidence that insulin resistance is likely to play a pathogenic role in DN onset-progression (7,8,20). To date, the ACE I/D polymorphism is the only gene that has been found to be related to the rate of DN progression (6,21). The impact of the DD ACE genotype on DN progression is of a similar degree (6) to that of the PC-1 Q allele.

All patients were on antihypertensive treatment, which primarily included ACE inhibitors, and there was no difference between the two PC-1 genotype groups in the type or duration of treatment (Table 1). Because these treatments slow the rate of progression of DN (1), it cannot be concluded that the faster rate of DN progression in the Q-carrying patients is the result of a reduced sensitivity to antihypertensive and/or ACE inhibitor therapy.

We are aware of the shortcomings of retrospective studies in investigating the rate of disease progression. However, data collection was carefully executed in this cohort of patients, and to date no prospective study of the genetic

determinants of DN progression is available. We believe, therefore, that properly conducted longitudinal retrospective studies, the present study among which is one of the largest, can still provide useful information.

In conclusion, our data show that the PC-1 Q121 amino acid variant is associated with a faster rate of DN progression in type 1 diabetic patients with albuminuria. PC-1 genotyping may help, therefore, in identifying fast progressor patients at an early stage of diabetic renal disease and target intensive therapy.

## RESEARCH DESIGN AND METHODS

**Patients.** Type 1 diabetes was defined as 1) disease onset before 30 years of age, 2) evidence of ketosis at diagnosis, and 3) the absolute need of continued insulin therapy within 6 months of diagnosis. DN was defined as the presence of persistent proteinuria at the beginning of the follow-up and evidence of diabetic retinopathy. Persistent proteinuria was diagnosed if, on at least two consecutive occasions, the following criteria were fulfilled: AER >300 mg/24 h and/or albumin-to-creatinine ratio >30 mg/mmol on morning urine sample ( $n = 55$ ) or a urine sample dipstick positive for protein (1+ or more) ( $n = 22$ ).

Of 175 European patients with type 1 diabetes and DN who were seen from January 1995 to April 1997 in the diabetes units of five centers (four in Italy and one in the U.K.), 81 patients fulfilled the following selection criteria for participating in the study: age <70 years, available medical information dating back 2 or more years, and measurements of serum creatinine on five or more occasions. DNA for PC-1 genotyping was available in 77 of these 81 patients (31 from the U.K. and 46 from Italy). The average follow-up was 6.5 years (range 2.5–15). At the time of sampling for DNA, all patients underwent a standard clinical examination, which included two measurements of blood pressure to the nearest 2 mmHg in the sitting position after at least a 5-min rest using a mercury sphygmomanometer and an appropriate sized cuff. Diastolic blood pressure was recorded at the disappearance of Korotkoff sound (phase V). MAP was calculated as diastolic plus one-third of the pulse pressure. Serum creatinine was measured using the Jaffé reaction-rate method (Hitachi 737 Autoanalyzer; Hitachi, Tokyo), glycated hemoglobin was measured by high-performance liquid chromatography (Diamat Analyzer; Bio-Rad, Richmond, CA), and urinary albumin concentration was measured in three timed 24-h urine collections by a nephelometric method (Behring Nephelometer Analyzer; Marburg, Germany). On average, serum creatinine measurements were available on an 8-month basis, and creatinine clearance was calculated with the Cockcroft-Gault formula (18) and used as a measure of GFR. To validate the Cockcroft-Gault formula as a measure of GFR, its predictive accuracy for GFR was assessed in a subset of 45 patients in whom simultaneous measures of  $^{51}\text{Cr}$ -EDTA GFR were available. GFR calculated with the Cockcroft-Gault formula correlated highly significantly with that measured by  $^{51}\text{Cr}$ -EDTA ( $r = 0.851$ ,  $P = 0.001$ ). For the GFR decline, analysis of the means of the differences of these two methods as proposed by Bland and Altman (22) indicated that differences were constant over the whole range of GFR (mean of the means of the differences:  $0.03 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ ; limits of agreement [mean  $\pm 2$  SD]:  $-3.02$  to  $3.08 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ ) (22).

All subjects gave their informed consent to participate in the study, which was performed according to the Declaration of Helsinki guidelines and with the approval of the local ethic committees.

**Genotyping.** High molecular weight DNA for genotyping was extracted from peripheral blood (5–10 ml) that was taken into EDTA-containing tubes, was frozen as whole, and was stored at  $-30^\circ\text{C}$  until extraction. Genomic DNA was extracted by proteinase K-phenol/chloroform standard method (17,23), resuspended in 10 mmol/l Tris-HCl, pH 8.0, and 1 mmol/l EDTA, and stored at  $4^\circ\text{C}$ . The polymerase chain reaction technique, specific primers, and experimental conditions used for genotyping have been previously described (17).

**Statistical analysis.** Data are reported as means ( $\pm$  SE) or median (range). Mean differences were compared by unpaired Student's *t* or Mann-Whitney *U* tests, as appropriate. A *P* value <0.05 was considered to be significant. Because part of this series has been studied to test the association of GFR decline with other genes in preliminary studies (24,25), adjustment for multiple comparisons was applied.

Two-way ANOVA on all measurements ( $n = 788$ ; 587 with an average of  $10.6 \pm 1.0$  observations per patient for KK subjects, and 201 with an average of  $9.1 \pm 2$  observations per patient for Q-carrying patients, respectively) of the change in GFR (GFR decline over the time) in each patient was used to compare different groups. Analysis was weighted for different lengths of follow-up and different numbers of assessment time points in different individuals. Individual changes in GFR values, which were measured at each time point (on average every 8 months), were used to calculate the individual rate of DN progression. Univariate and multiple variate analyses were used to correlate independent variables of progression with the dependent variable, that being the rate of GFR decline. For this analysis, AER values were logarithmically transformed.

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