The role of PC-1 and ACE genes in diabetic nephropathy in type 1 diabetic patients: evidence for a polygenic control of kidney disease progression

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Abstract

Background. The DD genotype of the ACE gene predisposes to faster diabetic nephropathy (DN) progression but its role in DN development is more controversial. We reported previously, in type 1 diabetic patients, an association between faster DN progression and the PC-1 gene Q121 variant, which associates with insulin resistance in non-diabetic subjects. We investigated here whether the combination of the ACE DD genotype and the PC-1 Q121 variant predicts the development and/or progression of DN in type 1 diabetic patients.

Methods. Type 1 diabetic patients either with (n = 159) or without (n = 122) nephropathy were evaluated in a cross-sectional study. DN was defined as the presence of microalbuminuria or persistent proteinuria in a subject with more than 10-year duration of disease and concomitant diabetic retinopathy, and with no evidence of heart failure or other renal disease. Seventy-five (47 male/28 female) type 1 diabetic patients with nephropathy in whom retrospective information with repeated measurements of serum creatinine was available, were analysed in a longitudinal study.

Results. No association of the PC-1 Q121 variant and the ACE D/D genotype with DN development was observed. However, the ACE DD genotype and the PC-1 Q121 variant were associated, both independently (P = 0.02 and P = 0.025, respectively) or in combination (P = 0.02), with a faster rate of glomerular filtration rate decline. An interaction (P = 0.03) was observed between the two genes in increasing the individual patient’s risk of being a fast progressor.

Conclusion. Our data suggest that, in type 1 diabetic patients, the ACE and the PC-1 genes interact in increasing the individual risk of having a faster DN progression.

Keywords: albuminuria; end-stage renal failure; gene–gene interaction; gene polymorphism; type 1 diabetes

Introduction

Diabetic nephropathy (DN) develops in less than about 40% of type 1 diabetic patients and its rate of progression varies greatly among individuals [1]. Several studies have demonstrated clearly that both environmental and genetic factors are implicated in the development and progression of DN [1]. The genetic background of DN is believed to be polygenic and several genes have been found to have small effects [2].

Insulin resistance characterizes type 1 diabetic patients who develop albuminuria as well as their non-diabetic first degree relatives [3] 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. These observations suggest that insulin resistance may precede and play a role in DN. As for DN, insulin resistance too has genetic determinants [4,5]. One could speculate, therefore, that insulin resistance and DN may share common genetic determinants. We have shown recently that the Q121
variant of PC-1, a gene which encodes for a membrane glycoprotein known to inhibit insulin signalling, is associated with insulin resistance in the general population [6–9] and with a faster progression of DN in type 1 diabetic patients [10].

The DD genotype of the ACE gene is a marker of higher ACE activity and mortality for coronary heart disease among diabetics [11]. In addition, the ACE DD genotype associates with insulin resistance in hypertensive but not in normotensive subjects [12]. Most patients with DN are hypertensive and the DD genotype has been found to be associated with faster DN progression both in type 1 [13] and in type 2 diabetes [14].

Whether the PC-1 and ACE genes act in combination with respect to DN development and/or progression is unknown. In this study we have examined the combined effect of the PC-1 Q121 variant and ACE DD genotype on the risk of DN development and progression in type 1 diabetic patients.

Subjects and methods

Cross-sectional study

The patients investigated are part of a cohort described previously [15]. Briefly, two groups of subjects were studied: type 1 diabetic patients with elevated urinary albumin excretion rate (AER) (DN, n = 159) and type 1 diabetic patients with a long duration of disease (greater than 15 years) and normal AER (diabetic control, DC, n = 122). Seventy per cent of subjects were recruited in Italy and the remainder in the UK. All subjects were white, of European origin, with grandparents born in Italy or in the UK and had to be less than 70 years old. Type 1 diabetes was defined as disease onset before 30 years of age, evidence of ketosis at diagnosis, and absolute need of continued insulin therapy within 6 months of diagnosis. The demographic and clinical features of the diabetic patients from Italy and UK were comparable (data not shown).

Patients with nephropathy

DN was defined as the presence of microalbuminuria or persistent proteinuria in a subject with more than 10 years duration of disease and concomitant diabetic retinopathy (background or proliferative), but no evidence of heart failure or other renal disease. Microalbuminuria was diagnosed if an AER was greater than 30 but lower than 300 mg/24 h or an albumin/creatinine ratio (ACR) was greater than 2.5 in males and 3.5 mg/mmol in females but lower than 30 mg/mmol on at least two consecutive occasions. Persistent proteinuria was diagnosed if an AER was greater than 300 mg/24 h or an ACR greater than 30 mg/mmol or a urine sample dipstick positive for protein (1+ or more) on at least two consecutive occasions. 159 patients satisfied the inclusion criteria and were genotyped for both ACE and PC-1 genes.

Patients without nephropathy

Absence of nephropathy was defined as an AER persistently (on three or more 24 h collections) within the normal range of less than 30 mg/24 h or ACR lower than 2.5 in males and 3.5 mg/mmol in females in a subject with more than 15 years of diabetes. Epidemiological studies suggest that the risk of developing nephropathy in this group of patients is low. One hundred and twenty-two patients satisfied the inclusion criteria and were genotyped for both ACE and PC-1 genes.

The clinical characteristics of the two groups of diabetic patients studied are shown in Table 1. In the DN group, 59 patients had microalbuminuria. No significant difference was seen between DC and DN patients in terms of age and duration of diabetes. As expected, there was a greater representation of males in the DN group, even though this difference did not reach statistical significance. DN patients had higher values of systolic and diastolic blood pressure, glycated haemoglobin, serum creatinine, total cholesterol, and triglycerides (P < 0.001 for all). Sixty per cent of DC patients had diabetic retinopathy. There were no significant clinical and biochemical differences between Italian and British patients (data not shown).

Out of the 159 type 1 diabetic patients with DN, 75 (47 males/28 females, age: 42 ± 10 years; 30 from the UK and 45 from Italy) patients fulfilled the following selection criteria for entering into the longitudinal study: information dating back 2 or more years, persistent proteinuria at baseline, measurements of serum creatinine on five or more occasions, and availability of DNA for genotyping.

The clinical features of the 75 type 1 diabetic patients with DN studied longitudinally are given in Table 2. There were no significant clinical and biochemical differences between Italian and British patients (data not shown). At the time of DNA sampling patients had a mean age of 42 years and a mean duration of diabetes of 26 years. All patients were receiving antihypertensive therapy of whom 95% were taking ACE inhibitors. As a result of antihypertensive therapy, the clinical characteristics of the two groups of diabetic patients with nephropathy are shown in Table 2. The prevalence of diabetes and other cardiovascular risk factors was comparable between the two groups of patients.

<table>
<thead>
<tr>
<th>Table 1. Clinical features of type 1 diabetic patients with or without nephropathy</th>
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<tbody>
<tr>
<td>DC</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Gender (male/female)</td>
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<tr>
<td>Duration of diabetes (years)</td>
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<tr>
<td>Glycated haemoglobin (%)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
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<tr>
<td>Plasma creatinine (μM)</td>
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<tr>
<td>Triglycerides (mM)</td>
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<tr>
<td>Total cholesterol (mM)</td>
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<td>HDL cholesterol (mM)</td>
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Data are mean ± SD or *median (range). *P < 0.001.

<table>
<thead>
<tr>
<th>Table 2. Clinical features of 75 type 1 diabetic patients with nephropathy</th>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Gender (male/female)</td>
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<tr>
<td>Duration of diabetes (years)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
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<tr>
<td>MAP (mmHg)</td>
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<tr>
<td>AER (mg/day)</td>
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<tr>
<td>Follow-up (years)</td>
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<td>95% on ACE-I therapy (years)</td>
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</tbody>
</table>

Data are mean ± SD or *median (range).
AER had fallen below 300 mg/24 h in 20% patients. Median serum creatinine was 97 mmol/l ranging from 55 to 504 mmol/l.

All patients were on antihypertensive medications (95% ACE inhibitors). 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 5 min rest using a mercury sphygmomanometer and an appropriately sized cuff. Diastolic blood pressure was recorded at the disappearance of Korotkoff sound (phase V). Mean arterial pressure (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), glycated haemoglobin by HPLC (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, Behring, Marburg, Germany). Serum creatinine measurements were available, on average, on an 8 monthly basis and creatinine clearance was calculated with the Cockcroft–Gault formula [16] and used as a measure of glomerular filtration rate (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 51Cr-EDTA GFR were available. GFR calculated by Cockcroft–Gault correlated highly significantly with that measured by 51Cr-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, indicated that differences were constant over the whole range of GFR (mean of the means of the differences 0.03 ml/min/year; limits of agreement (mean ± 2 SD) –3.02 to 3.08 ml/min/year) [17].

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

Genotyping

Determination of PC-1 and ACE gene polymorphisms was performed in a central Laboratory at Scientific Institute ‘Casa Sollievo della Sofferenza’ San Giovanni Rotondo, Italy, as described previously [6,15].

Statistical analysis

All data are reported as mean ± SD, mean (range), or median (range). Mean differences were compared by unpaired Student’s t or Mann-Whitney U tests, as appropriate, for two groups and by one-way ANOVA for more than two groups.

Two-way ANOVA on all measurements in each patient (n = 773, with an average of 10.7 ± 1.0 observations/patient) of delta GFR (GFR decline over the time) was used to compare different groups, taking into consideration different length of follow-up and different number of assessment time points in different individuals. Individual delta GFR values, measured at each time point (on average every 8 months), were used to calculate the individual rate of DN progression. Patients enrolled into the longitudinal study were defined as fast or slow progressors according to their individual rate of GFR decline (i.e. above or below the median value of the rate of GFR decline of the whole cohort, respectively). A χ² test was used to compare the distribution of ACE and PC-1 genotypes and the percentages of patients being fast progressors across different genotype combinations.

Univariate and multiple variate analyses were used to correlate independent variables of progression with the dependent variable, rate of GFR decline. For this analysis, AER values were logarithmically transformed. Interaction between genes was tested with GLM Univariate analysis, statistical packaged SPSS version 10 (SPSS Inc., Chicago, IL, USA).

Results

Cross-sectional study

In all groups the distribution of ACE and PC-1 genotypes were in Hardy–Weinberg equilibrium.

The distribution of the PC-1 genotypes was not different between DC and DN patients and this was also the case for the ACE genotypes, as reported previously [15]. Also, the distribution of concomitant ACE DD genotype and PC-1 Q121 variant was similar in both cases and controls (Table 3).

Similar results were obtained when patients with DN were considered separately according to the presence of microalbuminuria or macroalbuminuria or when these data were analysed by country (data not shown).

Longitudinal study

In the whole cohort, the median rate of GFR decline was 4.2 ml/min/year (range –3.8–16.6) and correlated with AER (P = 0.001) and MAP (P = 0.046) but not HbA1c, measured at genotyping. As shown previously in a series comprising most of the patients presented

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>ACE</th>
<th>PC-1</th>
<th>ACE + PC-1</th>
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<tr>
<td></td>
<td>DD, n (%)</td>
<td>ID II, n (%)</td>
<td>KK, n (%)</td>
</tr>
<tr>
<td>DN</td>
<td>62 (39)</td>
<td>76/21 (61)</td>
<td>111 (70)</td>
</tr>
<tr>
<td>DC</td>
<td>59 (48)</td>
<td>47/16 (52)</td>
<td>88 (72)</td>
</tr>
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</table>

χ² = 2.76, P = 0.3
χ² = 1.73, P = 0.4
χ² = 3.2, P = 0.001
here [10]. PC-1 gene Q121 variant carriers \((n=21, 19 \text{ KQ}, \text{two QQ})\) had a faster GFR decline than K121K \((n=54)\) patients [6.6 (range 0.16–16.6) vs 2.6 ml/min/year (range –3.8–16.0), \(P<0.001\)]. In multivariate analysis, the PC-1 genotype was correlated with GFR decline independently \((P=0.025)\) of AER and MAP.

A progressive increase in the rate of GFR decline was also observed in relation to the ACE genotype in the 75 nephropathic patients: II + ID \((n=45, 3.5 \text{ ml/min/year (range 0.8–16)})\) and DD \((n=30, 6.9 \text{ ml/min/year (range –3.8–16.6)}, P=0.03)\). The univariate relation between the ACE I/D polymorphism and GFR decline \((P=0.02)\) was no longer significant \((P=0.1)\) after adjusting for both AER and MAP.

To investigate the combined effect of the ACE and PC-1 genes on the rate of DN progression, we divided the whole cohort of 75 patients into four groups, according to the absence/presence of ACE DD genotype and/or PC-1 Q121 variant: group 1 \((n=33)\) patients with ACE II/ID and PC-1 K121K genotypes; group 2 \((n=12)\) patients with PC-1 Q121 variant and ACE II/ID genotypes; group 3 \((n=21)\) ACE DD and PC-1 KK genotype; and, finally, group 4 \((n=9)\) patients with PC-1 Q121 variant and ACE DD genotype. The rate of loss of kidney function increased progressively and significantly from groups 1 to 4 (Figure 1a, \(P<0.001\)).

In a multivariate analysis, the combination of ACE and PC-1 genotypes (groups 1–4) \((P=0.02)\), AER \((P=0.008)\), duration of diabetes \((P=0.01)\) but not MAP \((P=0.07)\), independently correlated with GFR decline.

The effect of the ACE DD genotype and PC-1 Q121 variant on the median value of the rate of GFR decline for each of the four groups seems to be additive. The sum of the net effect of the PC-1 Q121 variant on increased GFR decline (group 2 minus group 1, GFR fall 1.8 ml/min/year) with that of the ACE DD genotype (group 3 minus group 1, GFR fall 3.7 ml/min/year) gives an expected net effect on GFR fall of 5.5 ml/min/year, which is very similar to that actually observed in patients carrying both the Q121 variant and the DD genotype (i.e. group 4). In fact in these patients the net effect of the combined genotypes on the GFR fall in respect to patients carrying the KK/II + ID genotypes (i.e. group 1) is 5.9 ml/min/year (7.3 ml/min/year of group 4 minus 2.4 ml/min/year of group 1). However, when the individual patient’s risk of being a fast progressor was taken into account (i.e. rate of GFR decline above 4.2 ml/min/year, the median value of the whole cohort) a clear interaction \((P=0.03)\) between the two genes was observed. The simultaneous presence of both Q allele of PC-1 and DD genotype of the ACE gene (i.e. group 4) was, in fact, needed to significantly increase \((P<0.001)\) the individual risk of being a fast progressor when compared with KK PC-1 and II + ID ACE genotypes (i.e. group 1) (Figure 1b). In contrast, no difference was observed between groups 1, 2, and 3. The gene–gene interaction was independent of known environmental promoters including AER and blood pressure.

Discussion

This study shows that PC-1 and ACE genes have no effect, either alone or in combination, on the development of DN. Similar data on the PC-1 gene have been reported recently in a Danish population [18]. The possibility of a false negative result in the cross-sectional study on DN development needs to be taken into account. The subjects of the study were all white Europeans whose grandparents were born either in Italy or the UK. When analysis was stratified by country no significant differences were found between different groups and the Hardy–Weinberg equilibrium was confirmed for all genotypes in the whole group.
Thus, in spite of the multiple geographical origins of the patients, the risk of population stratification bias should have been limited in our study. Although there is little argument that association analyses should be undertaken in cohorts as large as possible, our cohort of patients was sufficiently large, of a size comparable with that of other major studies. The cohort of patients with DN (i.e. cases) included individuals with persistent microalbuminuria or macroalbuminuria and diabetic retinopathy. The inclusion of patients with microalbuminuria not only makes our findings more readily comparable with several other series in which the definition of nephropathy was also inclusive of microalbuminuria but also minimizes the possibility of selection bias due to survivor effect. The DC patients (i.e. controls) comprised individuals with normal urinary albumin excretion and a long duration of diabetes, a group unlikely to include patients who will later develop nephropathy. This represents a ‘complication-protected’ subset, which would be expected to maximize differences, if any, when compared with the ‘complication-susceptible’ nephropathy subset. Based on these considerations we believe that the lack of association of these two genes with DN development is unlikely to be a false negative. In contrast, PC-1 and ACE genes, both alone or in combination, appear to play a role in modulating the rate of disease progression in patients with established nephropathy. A clear interaction between the two genes was observed on increasing the individual patient risk of being a fast progressor. While both genes have been reported previously by us and others to be risk of being a fast progressor in type 1 diabetic patients. Larger, prospective studies are needed to confirm this preliminary observation.

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References


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