

ACE Gene Polymorphism as a Prognostic Indicator in Patients With Type 2 Diabetes and Established Renal Disease

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OBJECTIVE — To investigate whether the DD genotype is a predictor of mortality and of the decline in renal function in patients with type 2 diabetes and established nephropathy.

RESEARCH DESIGN AND METHODS — A total of 56 such patients of Maltese Caucasian descent were recruited, and their ACE genotype was determined. Serum creatinine was estimated approximately every 4 months. The glomerular filtration rate (GFR) was calculated according to the Cockcroft-Gault formula, and rate of change was determined by regression analysis.

RESULTS — The rate of change in calculated GFR was $-7.76 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ in those with the DD genotype ($n = 31$) and $-1.17 \text{ ml} \cdot \text{min}^{-1} \cdot \text{h}^{-1}$ in those with the ID or II genotype ($n = 25$) ($P < 0.01$). The 3-year mortality was 45.2% in the DD group compared with 20.0% in the ID/II group ($P < 0.05$).

CONCLUSIONS — The DD genotype of the ACE gene polymorphism is associated with a more rapid decline in renal function and higher mortality in type 2 diabetic patients with established nephropathy.

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Diabetic nephropathy is one of the major long-term complications of diabetes. However, it is impossible to predict which patients will develop this complication. Although duration of diabetes, blood pressure, and glycemic control are undoubtedly important, other factors are probably also involved. There is considerable evidence of familial clustering of nephropathy in type 1 diabetes (1–3). Data on Pima Indians (4), as well as our own data in patients of Maltese Caucasian descent (5), have shown that familial clustering also occurs in type 2 diabetic nephropathy. This suggests that genetic factors may be involved in the etiology of renal disease in both major types of diabetes.

One possible genetic factor that has

attracted much attention is the ACE gene. This contains a polymorphism comprised of the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence within intron 16 (6). The polymorphism has been associated with diabetic nephropathy in type 1 and type 2 diabetes by some but not all authors (7); however, two recent meta-analyses have confirmed an association of the DD genotype with diabetic nephropathy (8,9). The rate of decline of renal function has been shown to be more rapid in type 1 diabetic patients who have the DD genotype (10). Two studies in type 2 diabetes have had similar results (11,12).

Patients with diabetic nephropathy are known to have a high mortality,

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Abbreviations: GFR, glomerular filtration rate; LVH, left ventricular hypertrophy; PAI-1, plasminogen activator 1; PCR, polymerase chain reaction.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

mostly resulting from cardiovascular disease (13,14). Tarnow et al. (15) recently reported increased mortality in parents of type 1 diabetic patients with nephropathy compared with parents of type 1 diabetic patients without nephropathy, suggesting a genetic predisposition to the increased mortality observed in patients with diabetic nephropathy. The DD genotype of the ACE polymorphism has been associated with left ventricular hypertrophy (LVH) in the general population (16). It has also been associated with coronary artery disease in both type 1 (17) and type 2 (18,19) diabetes. In addition, it has been associated with sudden death in patients with hypertrophic cardiomyopathy (20) and with coronary artery disease in the general population by some authors (8,21) but not by others (22,23).

The aim of the present study was to determine whether the DD genotype of the ACE gene polymorphism has a prognostic significance in type 2 diabetic patients with nephropathy, considering both the rate of deterioration in renal function and mortality rates.

RESEARCH DESIGN AND METHODS

Unrelated type 2 diabetic patients with proteinuria who attended the Diabetes Clinic, St. Luke's Hospital, Malta were recruited. Consecutive patients with dipstick-positive proteinuria were examined by two 24-h urinary protein estimations, a urine culture, a urine microscopy, and an ultrasound examination of the kidneys. All patients were of Maltese Caucasian descent. The inclusion criterion was a urinary protein excretion $\geq 500 \text{ mg/24 h}$ in two consecutive collections. Patients with hematuria, pyuria, a positive urine culture, or an abnormal ultrasound (other than increased renal size) were excluded. A total of 56 patients met these criteria. Using the above criteria, three patients were excluded (one with chronic pyelonephritis and two with nephrolithiasis). The age (mean \pm SD) of the study patients was 60.8 ± 12.1 years. The median (interquartile range) duration of diabetes

Table 1—Patient and clinical characteristics at entry into the study

	DD genotype	ID/II genotype	P
n	31	25	
Men, n (%)	21 (67.7)	15 (60.0)	0.55
Age in years, mean (SD)	62.6 (9.3)	58.5 (14.6)	0.22
Duration of diabetes, median (IQ range)	12 (3–22.5)	11 (4–16)	0.95
Urinary protein excretion in mg/24 h, median (IQ range)	1128 (722–3,962)	1288 (301–4,095)	0.89
Use of antihypertensive drugs, n (%)	27 (87.1)	22 (88.0)	0.92
Use of ACE inhibitor, n (%)	25 (80.6)	21 (84.0)	0.75
SBP in mmHg, mean (SD)	132.5 (15.8)	136.6 (17.3)	0.36
DBP in mmHg, mean (SD)	80.5 (6.8)	80.7 (8.7)	0.93
HbA _{1c} , mean (SD)	8.16 (1.37)	8.61 (1.92)	0.33
Serum cholesterol in mmol/l, mean (SD)	6.66 (1.59)	6.25 (2.00)	0.37
Serum triglycerides in mmol/l, median (IQ range)	1.94 (0.94–3.76)	1.62 (1.22–2.48)	0.79
Smokers, n (%)	9 (29.0)	6 (24.0)	0.67
Baseline calculated GFR, median (IQ range)	58.9 (31.9–65.7)	50.4 (33.2–61.9)	0.53

DBP, diastolic blood pressure; IQ, interquartile; SBP, systolic blood pressure.

from diagnosis was 12.0 years (3.5–20.5), and the median (interquartile range) urinary protein excretion was 1,160 mg/24 h (656–4,095); 50 (89.3%) patients had retinopathy.

DNA analysis

DNA was extracted from leukocytes using Nucleon II extraction technique (Tepnel, Glasgow). The ACE genotype was determined by polymerase chain reaction (PCR) using primers as described by Ohno et al. (24). The PCR was performed in a final volume of 25 μ l that contained 100 ng genomic DNA, 25 pmol/l of each primer, 200 μ mol/l dNTP, 0.5 units Amplitaq Gold DNA polymerase (Applied Biosystems, Warrington, U.K.), and 2 mmol/l MgCl₂. DNA was amplified by an initial hot-start at 95°C for 15 min, followed by 35 cycles consisting of denaturing at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min. This was followed by final extension at 72°C for 10 min. The PCR products were directly visualized with ethidium bromide staining after electrophoresis in a 2% agarose gel. Because preferential amplification of the D allele has reportedly resulted in possible mistyping of ID as DD (25), we also used an I-specific amplification method (24) on all samples initially typed as DD.

The outcome measures were 3-year all-cause mortality and rate of change in calculated glomerular filtration rate (GFR). Serum creatinine and other follow-up parameters were determined approximately every 4 months, and GFR

was calculated according to the Cockcroft-Gault formula (26). All patients were followed-up for 3 years or until death, whichever came first. There were no dropouts.

Statistical methods

The rate of change in GFR was determined by regression analysis. Student's *t* test was used to determine significant differences between means of normally distributed data. Skewed data (duration of diabetes and urinary protein excretion) were log-transformed before Student's *t* test was applied. Wilcoxon's rank-sum test was used to determine significant differences between other data that were not normally distributed (rate of change in GFR). The Z test was used to determine significant differences between percentages. Hardy-Weinberg equilibrium was assessed by comparing observed frequencies with expected frequencies using the χ^2 test. The relation between serum cholesterol, triglycerides, smoking, HbA_{1c}, and systolic and diastolic blood pressure to change in GFR was assessed by regression analyses.

RESULTS— A total of 31 patients had the DD genotype, 22 had the ID genotype, and 3 had the II genotype. This was in Hardy-Weinberg equilibrium ($\chi^2 = 0.13$, NS). Table 1 shows that the patients' initial clinical and biochemical characteristics did not vary according to ACE genotype status. The mean \pm SD of HbA_{1c} during follow-up was 8.19 \pm 1.26% in the DD group compared with 8.83 \pm

1.29% in the II/ID group (*P* = 0.06). The mean \pm SD for serum cholesterol during follow-up was 5.94 \pm 0.64 mmol/l in the DD group compared with 6.10 \pm 0.97 mmol/l in the II/ID group (*P* = 0.48), and the median (interquartile range) serum triglycerides was 2.05 mmol/l (1.05–3.84) in the DD group compared with 1.85 mmol/l (1.42–2.78) (*P* = 0.68) in the II/ID group. The mean \pm SD for systolic and diastolic blood pressure was 139.3 \pm 12.2 and 82.6 \pm 5.45 mmHg, respectively, in the DD genotype group compared with 144.4 \pm 14.4 (*P* = 0.15) and 83.3 \pm 7.26 mmHg, respectively, in the II/ID genotype (*P* = 0.06).

The rate of change in calculated GFR in individual patients is shown in Fig. 1. The median (interquartile range) rate of change of calculated GFR was -7.76 ml \cdot min⁻¹ \cdot year⁻¹ (-12.08 to -3.88) in those with the DD genotype and -1.17 ml \cdot min⁻¹ \cdot year⁻¹ (-2.17 to -0.39) in those

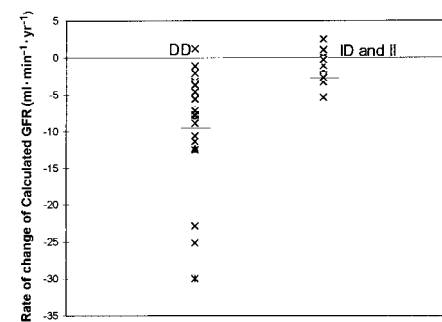


Figure 1—Data points showing change of calculated GFR according to ACE genotype. The horizontal lines refer to the median values.

Table 2—Clinical and biochemical parameters in those who died and those who were alive at 3 years

	Survivors	Fatalities	P
n	19	37	
SBP (mmHg)	131 (15)	140 (20)	0.08
DBP (mmHg)	80 (7)	83 (9)	0.20
HbA _{1c}	8.0 (1.2)	9.0 (2.3)	0.07
Serum cholesterol (mmol/l)	6.50 (1.69)	6.01 (1.13)	0.20

Data are means (SD).

with the ID/II genotype ($P < 0.01$). Rate of change in calculated GFR was not correlated with age ($P = 0.93$), HbA_{1c} ($P = 0.31$), serum cholesterol ($P = 0.89$), systolic blood pressure ($P = 0.45$), diastolic blood pressure ($P = 0.40$), or duration of diabetes ($P = 0.99$).

The 3-year all-cause mortality in the whole group was 19/56 (33.9%). The majority of these deaths (68.4%) were due to cardiovascular causes. The survival curves of the two groups is shown in Fig. 2. The 3-year mortality (95% CI) was 45.2% (29.2–62.2) in those with the DD genotype ($n = 31$) compared with 20.0% (4.3–35.7) in those with the ID/II genotype ($n = 25$) ($P = 0.04$). The difference (95% CI) in mortality between the two groups was 25.2% (1.7–48.7). Only three deaths were due to end-stage renal failure (one DD and two ID/II); therefore, the difference in mortality did not directly reflect the difference in rate of decline of GFR. The 3-year cardiovascular mortality (95% CI) was 32.3% (15.8–48.7) in the DD group compared with 12.0% (0.0–24.7) in the ID/II group ($P = 0.055$). The median (interquartile range) in rate of change in calculated GFR was $-3.50 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ (-13.42 to 1.26) in DD

patients who were alive at 3 years ($n = 17$) compared with $-0.45 \text{ ml} \cdot \text{min}^{-1} \cdot \text{year}^{-1}$ (-9.42 to 4.62) in those who were dead at 3 years ($n = 14$) ($P = 0.07$). Mortality was 27.8% (13.1–42.4) in men ($n = 36$) and 45.0% (23.2–66.8) in women ($P = 0.20$). Table 2 compares survivors' fatalities.

CONCLUSIONS— We have shown that the DD genotype of the ACE polymorphism predicts a poorer outcome in type 2 diabetic patients with nephropathy. It is not only associated with a more rapid decline in GFR but is also associated with a higher mortality rate.

The association of the DD genotype with a faster deterioration of GFR in Maltese Caucasians with type 2 diabetic nephropathy is consistent with previous studies. In type 1 diabetes, the DD genotype has been associated with a more rapid decline in renal function (27–29). Similar results have been seen in type 2 diabetes in Japan (11) and Finland (12) as well as in nondiabetic renal disease (29). Moreover, an excess of the DD genotype has been reported in type 2 diabetic patients on hemodialysis (30). More rapid deterioration in renal function is expected in the presence of the DD genotype because it has been shown to be associated with higher levels of ACE when compared with DI and II (8,31). The use of ACE inhibitors that lower ACE levels has been shown to be associated with a reduced rate of deterioration of GFR (32). The DD genotype might adversely affect the natural history of patients with diabetic nephropathy directly, or it might diminish the protective effect of ACE inhibitors, given the high proportion of our patients (82%) who were on these drugs.

We have shown that survival is worse in those with the DD genotype. This has not, to our knowledge, been previously reported in type 2 diabetic nephropathy.

The survival disadvantage of the DD genotype in our patients does not seem to be related to the more rapid decline in renal function because the main cause of death in our patients was, as expected, cardiovascular. Cambien et al. (21) reported the association of the DD genotype with myocardial infarction, and the association with coronary disease has been replicated in an exclusively female population (33) as well as in type 2 diabetic patients (19). Furthermore, a meta-analysis of almost 50,000 patients found the DD genotype to be associated with coronary artery disease and myocardial infarction (8). However, this has not been confirmed by large studies (22,23,34). The D allele has also been associated with increased risk of cardiovascular events after myocardial infarction in a Japanese population (35) and with mortality associated with heart failure in Caucasians (36). In contrast, Schachter et al. (37) reported that the DD genotype is more common in centenarians than in younger patients. It is possible that the genotype may confer a survival disadvantage in certain circumstances only or in particular patient groups and may even be advantageous in other instances. One might expect a lower mean age in those with the DD genotype in view of their higher mortality, but this was not seen in our study, in which the average age was similar in both groups. It is possible that the DD genotype is associated with a higher mortality in established diabetic renal disease only.

The mechanism by which the DD genotype is associated with increased mortality and cardiovascular disease is unknown. Our data suggest that the increased mortality is not mediated by the more rapid decline in renal function because only a few deaths were due to renal failure and the rate of decline was not higher in those who died. The DD genotype is associated with higher levels of circulating ACE (8,31). Blood ACE levels have been associated with LVH (38) and increased plasminogen activator 1 (PAI-1) activity (39); in addition, pharmacological ACE inhibition can reverse LVH (40,41) and arteriolar changes (42,43), decrease PAI-1 activity (39,44,45), and bring about alterations in renal hemodynamics (46–48). The D allele has been associated with LVH by some authors (16,49–51) but not by others (52–54) as well as with decreased re-

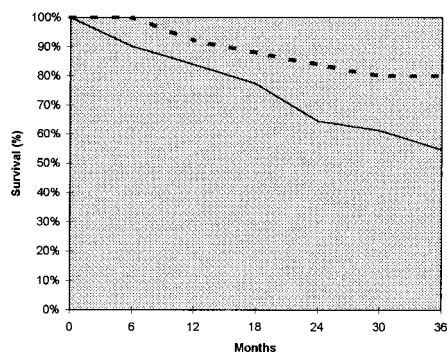


Figure 2—Kaplan-Meier survival curves for patients with the DD (—) and ID/II (---) genotypes.

gression of LVH in patients on ACE inhibitors (55). It has also been associated with increased PAI-1 levels (56,57) and endothelial dysfunction (58). These factors may all contribute to the decreased survival and higher rate of decline in renal function observed in patients with the DD genotype. This probably occurs independently of blood pressure, which was very similar in patients with the DD genotype compared with other patients (II or ID) in our study, although clinic readings were used rather than the more accurate 24-h monitoring values. It is noteworthy that Gharavi et al. (59) reported that the DD genotype is associated with increased left ventricular mass independently of blood pressure in hypertensive patients. Also, in a meta-analysis by Staessen et al. (8), the D allele was found to be a marker of atherosclerotic cardiovascular disease but not of hypertension. Furthermore, there is evidence that patients with the DD genotype are less susceptible to the renoprotective (antiproteinuric) effects of ACE inhibitors (60). Therefore, it is possible that DD patients might benefit from higher doses of ACE inhibitors even if their blood pressure is well controlled. We feel that this merits further study.

In view of the small number of patients in our study, our data need to be replicated by other investigators. However, our patient groups were well-defined and well-matched at baseline. Furthermore, our findings of increased rate of decline in renal function and increased mortality associated with the DD genotype are internally consistent as well as consistent with the published literature. We believe the reason we have been able to detect a difference in mortality despite our small sample size is because the high age of subjects at entry meant that the absolute mortality rate was much higher than in other studies. Studies in type 1 diabetes would require much larger numbers and longer follow-up to have sufficient power to detect a difference in mortality. We used the Cockcroft-Gault formula to calculate GFR. Although this has its limitations, it has the advantage of reproducibility without the use of cumbersome techniques. This is important in view of the need for repeated measurements.

Finally, our data suggest that the failure of some case-control studies to demonstrate an association between the DD genotype and diabetic nephropathy may be attributed to survival bias. If DD pa-

tients with nephropathy have increased mortality, then the incidence will be under-represented in cohorts of surviving subjects with type 2 diabetic nephropathy collected from clinics. This would reduce the apparent prevalence of the DD allele in affected subjects in a case-control study and reduce the likelihood of detecting an association.

In conclusion, we have demonstrated that the DD genotype of the ACE polymorphism is associated with a more rapid decline in calculated GFR and with an increased mortality in type 2 diabetic patients with established renal disease. If this is confirmed in larger studies and in other populations, ACE genotyping might be useful in selecting patients with a poorer prognosis and result in more aggressive treatment.

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