Effect of Angiotensin-Converting Enzyme (ACE) Gene Polymorphism on Progression of Renal Disease and the Influence of ACE Inhibition in IDDM Patients

Findings From the EUCLID Randomized Controlled Trial

Guiseppe Penno, Nish Chaturvedi, Philippa J. Talmud, Patricia Cotroneo, Andrea Manto, Monica Nannipieri, Le-Ahn Luong, John H. Fuller, and the EUCLID Study Group

We examined whether the ACE gene insertion/deletion (I/D) polymorphism modulates renal disease progression in IDDM and how ACE inhibitors influence this relationship. The EURODIAB Controlled Trial of Lisinopril in IDDM is a multicenter randomized placebo-controlled trial in 530 nonhypertensive, mainly normoalbuminuric IDDM patients aged 20–59 years. Albumin excretion rate (AER) was measured every 6 months for 2 years. Genotype distribution was 15% II, 58% ID, and 27% DD. Between genotypes, there were no differences in baseline characteristics or in changes in blood pressure and glycemic control throughout the trial. There was a significant interaction between the II and DD genotype groups and treatment on change in AER (P = 0.05). Patients with the II genotype showed the fastest rate of AER progression on placebo but had an enhanced response to lisinopril. AER at 2 years (adjusted for baseline AER) was 51.3% lower on lisinopril than placebo in the II genotype patients (95% CI, 15.7 to 71.8; P = 0.01), 14.8% in the ID group (–7.8 to 32.7; P = 0.2), and 7.7% in the DD group (–36.6 to 37.6; P = 0.7). Absolute differences in AER between placebo and lisinopril at 2 years were 8.1, 1.7, and 0.8 μg/min in the II, ID, and DD groups, respectively. The significant beneficial effect of lisinopril on AER in the II group persisted when adjusted for center, blood pressure, and glycemic control, and also for diastolic blood pressure at 1 month into the study. Progression from normoalbuminuria to microalbuminuria (lisinopril versus placebo) was 0.27 (0.03–2.26; P = 0.2) in the II group, and 1.30 (0.33–5.17; P = 0.7) in the DD group (P = 0.6 for interaction). Knowledge of ACE genotype may be of value in determining the likely impact of ACE inhibitor treatment. ***Diabetes*** **47:1507–1511, 1998**

From the Cattedra di Malattie Metaboliche e del Ricambio (G.P., M.N.), Presidio Ospedaliero di Cisanello Diabetologia, Pisa; Instituto di Clinica Medica (P.C., A.M.), Universita Cattolica del Sacre Cuore, Rome, Italy; the Division of Cardiovascular Genetics (P.J.T., L.-A.L.), Rayne Institute, and EURODIAB (N.C., J.H.F.), University College London, London, U.K.

Address correspondence and reprint requests to Dr. Nish Chaturvedi, EURODIAB, University College London, 1-19 Torrington Place, London, U.K.

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AER, albumin excretion rate; D, deletion; EUCLID, EURODIAB Controlled Trial of Lisinopril in IDDM; I, insertion.

Only a third to a quarter of all patients with IDDM develop advanced diabetic nephropathy (1). Observations of a high concordance for nephropathy in families with multiple IDDM siblings indicate that hereditary factors may be important in its pathogenesis (2). A clear candidate for this role is the diallelic (insertion/deletion [I/D]) polymorphism in the ACE gene. This polymorphism is correlated to plasma ACE concentration (3), and ACE activity is significantly elevated in diabetic subjects with nephropathy (4,5). However, studies examining the role of the ACE gene in the pathogenesis of IDDM-related nephropathy have yielded contradictory results (4–8). These previous studies have been cross-sectional in nature and cannot fully assess causal relationships.

It is now widely recognized that ACE inhibitors can retard the progression of diabetic renal disease in a manner that may be independent of their effects on systemic blood pressure (9,10). However, the favorable effect of ACE blockade on the progression of diabetic nephropathy is variable, which may be partly due to genetic factors. Again, one such factor is the I/D polymorphism of the ACE gene, but studies have produced conflicting findings, with some indicating that patients homozygous for the D allele were more likely to respond to ACE inhibitor therapy (11,12), and some indicating the reverse (13,14). Only one study has examined this effect in people with IDDM (14); however, it was an observational study, with only 35 patients receiving ACE inhibitor therapy, and no comparison was made with a placebo group to assess the full impact of ACE inhibition.

We therefore tested the hypothesis that the I/D polymorphism of the ACE gene modulates the therapeutic effect of ACE inhibition on progression of urinary albumin excretion in IDDM patients using data from a randomized controlled trial (15).

RESEARCH DESIGN AND METHODS

The EURODIAB Controlled Trial of Lisinopril in IDDM (EUCLID) study is a multicenter, prospective, randomized, placebo-controlled trial of lisinopril, an ACE inhibitor, on albumin excretion rate (AER) in nonhypertensive IDDM patients with normoalbuminuria (85%) or microalbuminuria (15%). Full details of the study have been published previously (15), but in brief, 530 IDDM patients aged 20–59 years

TABLE 1
Baseline characteristics by ACE genotype

	II	ID	DD	*P* value
n (%)	77 (15)	296 (58)	137 (27)	
M/W	46/31	176/120	86/51	0.8
Age (years)	35 ± 9	34 ± 9	35 ± 9	0.3
Duration (years)	15 ± 7	14 ± 8	16 ± 9	0.06
BMI (kg/m^2)	24.7 ± 2.8	24.6 ± 3.0	24.7 ± 2.7	0.9
Systolic blood pressure (mmHg)	122 ± 12	122 ± 11	123 ± 11	0.9
Diastolic blood pressure (mmHg)	80 ± 5	80 ± 4	81 ± 4	0.6
AER (µg/min)	9.2	7.6	8.1	0.4
Interquartile range	5.2–16.6	4.1–13.2	4.7–13.5	
Microalbuminuria (%)	19	13	17	0.3
HbA_{1c} (%)	7.3 ± 1.9	7.2 ± 1.9	7.2 ± 2.1	0.9
Placebo/lisinopril	48/29	142/154	66/71	0.07

Data are means ± SD, or prevalence.

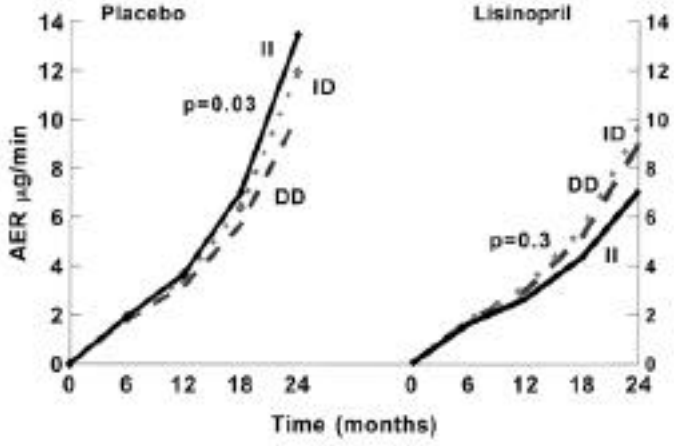

FIG. 1. Absolute increase in AER (µg/min) over a period of 2 years by ACE genotype in placebo and lisinopril groups. *P* values are for differences in AER change between II and DD ACE genotypes, adjusted for baseline AER.

were recruited from 18 European centers. Diastolic blood pressure was between ≥75 and ≤90 mmHg, and systolic blood pressure was ≤155 mmHg. The follow-up period was 2 years, and blood and urine samples were collected every 6 months. Patients provided two overnight urine collections for each estimation, and aliquots were sent to a central laboratory for albumin measurement using an immunoturbidimetric assay (16). Blood samples were sent to a central laboratory for the estimation of HbA_{1c} using an enzyme immunoassay with monoclonal antibody raised against HbA_{1c} (normal range, 2.9–4.8%) (Dako, Ely, Cambridgeshire, U.K.) (17). Treatment was started at 10 mg of lisinopril or matching placebo. At 3 months, the dose of medication could be increased to 20 mg if diastolic blood pressure did not fall below the target level of 75 mmHg.

Determination of the ACE genotype. Whole blood samples were taken at baseline and were frozen and sent for genetic analysis to University College London. Lymphocytes were isolated, and DNA was prepared by quick preparation from 100 µl of blood (18). The I/D polymorphism in intron 16 of the ACE gene was assessed by polymerase chain reaction using conditions previously described (19). To avoid mistyping heterozygotes as DD because of the preferential amplification of the D allele as compared with the I allele, DNA from all subjects with the DD genotype was amplified again, using an I-specific primer (5′ TTTGAGACGGGAGTCTCGCTC 3′) (20). Suitable samples for genetic analysis were available for 510 (96%) of 530 patients.

Statistical analysis. Data are expressed as means ± SD. Because urinary albumin excretion has a positively skewed distribution and was effectively log-transformed for analysis, its values were given as geometric means (25th, 75th percentile). The change in AER, the main end point of the study, was calculated for each patient by a summary measures approach, described previously (15). In this approach, the slope of the regression line for AER is calculated for each individual using the regression through the origin approach. The baseline AER measurement is not included in the calculation of the slope, but adjustment is made for it in the multivariate model.

For this variable and for normally distributed continuous variables, groups were compared by analysis of covariance, with adjustment made for confounders. Treatment by genotype interactions were tested, as were interactions for diabetes duration, split at 14 years, and HbA_{1c}, split at 7% (the median) by genotype within treatment group. Only those patients who had had a baseline assessment of AER with at least one follow-up assessment could be included in analyses examining change in AER; thus, out of 510 patients, 479 (73 II, 281 ID, and 125 DD) patients had analyzable data. Categorical variables were compared using the χ^2 test. In these analyses, comparisons between genotypes have been restricted to the homozygous groups (II versus DD). Progression to macroalbuminuria was defined as an AER >200 µg/min at the final visit, or at the last visit attended in those with microalbuminuria at baseline. Similarly, progression to microalbuminuria was defined as an AER between 20 and 200 µg/min in those with normoalbuminuria at baseline. Regression to normoalbuminuria was also compared in those with microalbuminuria at baseline. Odds ratios and precision-based CIs were calculated according to the method of Woolf (21). Of the 510 patients with genotype data available, 3 had no AER measurement at baseline, and a further 6 patients (5 in the ID group and 1 in the DD group) were excluded from the analysis of progression and regression because they were macroalbuminuric at baseline, thus leaving 501 patients for this analysis.

RESULTS

The distribution of the ACE gene I/D polymorphism was 15% II (n = 77), 58% ID (n = 296), and 27% DD (n = 137). Baseline clinical characteristics did not differ significantly by ACE genotype (Table 1). In the whole study population, HbA_{1c} barely changed, and diastolic blood pressure was lower by 3 mmHg in the lisinopril group compared with the placebo group, by 1 month into the study and for the rest of the study period (15). These changes were similar by genotype.

Baseline characteristics, including ACE genotype distribution (P = 0.3), did not differ between those for whom follow-up data on AER were available (n = 479) and those for whom only baseline data were available (n = 31). In the placebo group, patients with the II genotype had the largest increase in AER, and those with the DD genotype had the smallest increase (P = 0.03 for comparison between the II and DD groups) (Fig. 1). However, in the lisinopril-treated group, the AER increase was smallest in the II group and largest in the ID and DD groups (P = 0.3).

The effect of treatment on change in AER was significant (P = 0.02), while that for genotype was not (P = 0.8). The interaction test between treatment and ACE genotype, comparing patients in the II or DD group, was significant at P = 0.05. Considering the whole cohort and disregarding genotype, the absolute (2.2 µg/min) and relative (18.8%) difference in AER between placebo and lisinopril groups at 2 years was significant (P = 0.03). The relative treatment difference in AER at 2 years by genotype was as follows: mean AER was 51.3% (15.7 to 71.8; P = 0.01) lower on lisinopril than on placebo in the II group, 14.8% (–7.8 to 32.7; P = 0.2) in the ID group, and 7.7% (–36.6 to 37.6; P = 0.7) in the DD group. In absolute terms, the mean AER was 8.11, 1.70, and 0.78 µg/min lower in the lisinopril group than in the placebo group at 2 years in the II, ID, and DD groups, respectively (Fig. 2).

The significant relative treatment difference in AER of 51.3% in the II group persisted when further adjustment was made for the potential confounders center, sex, baseline diastolic blood pressure, and glycemic control, giving a treatment difference in AER of 60.3% (22.7 to 79.6; P = 0.009). We also adjusted this final model for diastolic blood pressure at 1 month to assess whether the treatment effect could be due

to the effects on blood pressure itself. This adjustment had little effect on the final treatment difference, which was 60.6% (20.3 to 80.6; $P = 0.0009$). Substitution of systolic for diastolic blood pressure made no difference in the final treatment effect. Change in AER did not differ according to diabetes duration in either the placebo ($P = 1.0$) or lisinopril ($P = 0.8$) groups. Furthermore, there was no evidence of an interaction between genotype and diabetes duration ($P = 0.6$ for both placebo and lisinopril groups). There was a difference according to HbA_{1c}, with those patients with worse control progressing faster in the placebo ($P = 0.01$) and lisinopril ($P = 0.07$) groups, but again there was no evidence for an interaction with genotype ($P = 0.7$ in the placebo group and $P = 0.8$ in the lisinopril group).

Progression from normoalbuminuria to microalbuminuria was favorably reduced on treatment in the II group (odds ratio, 0.27; $P = 0.2$) compared with the DD group (1.30; $P = 0.7$); however, the test for interaction between these groups was not significant ($P = 0.6$) (Table 2).

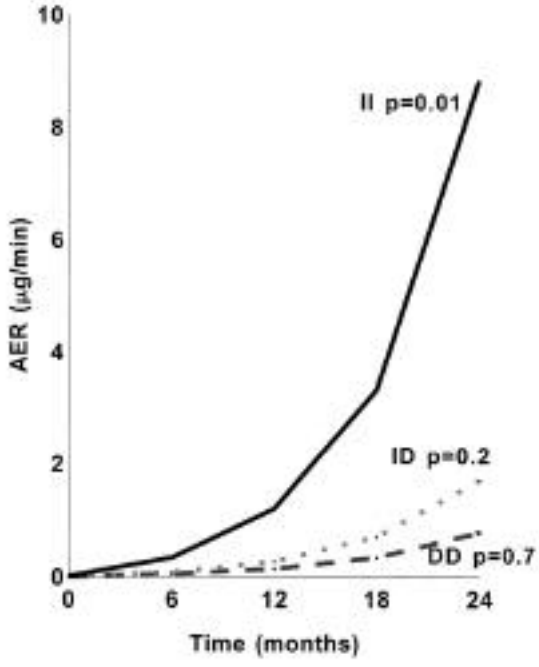

FIG. 2. Absolute difference (placebo – lisinopril) in AER (µg/min) over a period of 2 years by ACE genotype. *P* values are for treatment differences for changes in AER.

DISCUSSION

Patients homozygous for the I polymorphism of the ACE gene in the placebo group had the greatest rate of progression of AER in the placebo group. However, we also show that the prevalence of microalbuminuria was similar by genotype. These contrasting findings illustrate the difficulty of relying on cross-sectional data alone for interpretation. Our findings run counter to other observations, which either indicate that the D allele is the nephropathy gene (4,6,22) or show no association (5,7,8,23). Earlier cross-sectional studies cannot take the speed of disease progression into account, and biases in sample selection may thus result in an overrepresentation of a particular genotypic group. Furthermore, given that the response to ACE inhibition also differs by ACE genotype, a biased estimate of the relationship between diabetic nephropathy and ACE gene polymorphism might also result if the ACE genotype is not taken into account. In a previous, smaller study designed to examine the effect of ACE genotype on AER progression in IDDM, AER was observed to be higher in the DD group compared with the combined II and ID groups (14). We demonstrate that there may be difficulties in combining the latter two groups, inasmuch as the II group had the highest AER and highest prevalence of microalbuminuria and the ID group had the lowest AER and the lowest prevalence of microalbuminuria. If we had combined these groups, we, too, would have observed that the DD group had the highest AER, a finding that could be misleading. Interestingly, a more sophisticated family study showed that transmission of the I allele exceeded 50% in probands with diabetic nephropathy and that this effect was similar in people with long- and short-duration diabetes, a finding compatible with those of the EUCLID study (24).

Patients homozygous for the II allele also demonstrated the greatest response to treatment with ACE inhibitors. The favorable effect of ACE inhibition in slowing the progression of AER in patients with the II genotype cannot be explained by differences in blood pressure changes or by other known prognosticators such as glycemic control.

TABLE 2
Treatment effect on change in albuminuric status by ACE genotype

Type of change	Genotype	Change status/at risk		Odds ratio (95% CI)	*P* value
		Placebo	Lisinopril		
Progression to macro- from microalbuminuria	II	2/11	0/4	0.42 (0.02–10.75)	0.4
	ID	4/14	3/23	0.38 (0.07–1.99)	0.2
	DD	0/9	0/14	— (—)	
	Total	6/34	3/41	0.37 (0.08–1.55)	0.2
Progression to micro- from normoalbuminuria	II	5/37	1/25	0.27 (0.03–2.18)	0.2
	ID	7/125	6/126	0.84 (0.27–2.56)	0.8
	DD	4/57	5/56	1.30 (0.33–5.13)	0.7
	Total	16/219	12/207	0.78 (0.36–1.68)	0.5
Regression to normo- from microalbuminuria	II	4/11	4/4	15.0 (0.65–348.9)	0.08
	ID	4/14	10/23	1.92 (0.46–8.06)	0.4
	DD	3/9	5/14	1.11 (0.18–6.75)	0.9
	Total	11/34	19/41	1.81 (0.70–4.66)	0.2

Previous studies have provided conflicting evidence on the relationship between ACE gene polymorphism and renal response to ACE inhibitors. Among people with immunoglobulin A nephropathy (11) or with proteinuria of unknown origin (12), those homozygous for the DD allele demonstrated a greater beneficial effect of ACE inhibitors on renal disease than those without this allele. These observations have led others to recommend the use of ACE inhibitors for people with the DD genotype, in particular (25). However, our EUCLID findings confirm those from smaller observational studies in people who already have nephropathy (14) or nondiabetic renal disease (13,26). All these studies show that people homozygous for the D allele are less responsive to ACE inhibitors than those in other genotype groups.

The mechanism for this association is unknown (27); however, there are certain clues. Any hypothetical mechanism must be independent of blood pressure because almost all studies failed to show an association between the I/D polymorphism and hypertension (28). However, tissue and circulating ACE activity are under tight genetic control (27), with the II genotype being associated with the lowest activity and DD with the highest. So how can we account for the apparent dissociation between the genetic influence on ACE activity, on the one hand, and the response to ACE inhibition, on the other? One possible explanation is that serum ACE levels may not necessarily reflect cellular ACE activity, although this explanation does not appear to apply to cells such as circulating T-cells (29), and such a complete dissociation needed to account for our findings is hard to envisage. A second, more likely possibility is that the effect of ACE genotype occurs via mechanisms other than its effect on serum ACE levels. In support of this last hypothesis is the finding from the EUCLID study that ACE inhibitors also have a beneficial effect on diabetic retinopathy (30), even though retinopathy, unlike nephropathy, is not associated with elevated ACE levels (4,5).

In conclusion, the EUCLID study indicates that the ACE gene I/D polymorphism may be a significant determinant of the renoprotective efficacy of ACE inhibitor treatment in IDDM patients. We suggest that while those with the II genotype have a greater rate of progression of AER in the absence of ACE inhibitor treatment, patients in this group also have the greatest response to ACE inhibitors in terms of slowing the progression of AER, and those with the DD genotype are the most resistant. If confirmed, our findings have important implications for care provision and for further research. The identification of IDDM patients with the II genotype may be of value in that ACE inhibitor therapy can be instituted relatively early in the course of renal disease. In contrast, further work needs to be undertaken to confirm these findings and to assess whether patients with the DD genotype will respond at all to ACE inhibitor therapy, even when high doses are prescribed. This difference in treatment response based on genotype underlines the need to continue the search for other treatments designed to reduce the impact of renal disease in diabetes.

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APPENDIX: EUCLID INVESTIGATORS

B. Karamanos, A. Kofinis, C. Petrou, Hippokration Hospital, Athens, Greece; C. Ionescu-Tirgovisite, C. Iosif, Clinic of Diabetes Nutrition and Metabolic Diseases, Bucharest, Romania;

G. Tamas, G. Bibok, 1st Dept. Medicine Semmelweis University, Budapest, Hungary; Z. Kerenyi, P. Kis-Gombos, J. Toth, Szent Imré Teaching Hospital, Budapest, Hungary;

B. Ferriss, G. Grealy, Cork Regional Hospital, Cork, Ireland; R. Rottiers, H. Priem, University Hospital, Gent, Belgium; V. Koivisto, J. Touminen, E. Kostamo, Helsinki University Hospital, Helsinki, Finland; B. Idzior-Walus, B. Solnica, D. Galicka-Latalie, University School of Medicine, Krakow, Poland; G. Michel, M. Keipes, A. Giuliani, A. Herode, Centre Hospitalier de Luxembourg, Luxembourg; F. Santeusanio, A. Bueti, S. Bistoni, D. Cagini, Instituto di Medicina Interna e Scienze Endocrine e Metaboliche, Perugia, Italy; R. Navalesi, G. Penno, M. Nannipieri, L. Rizzo, S. Bandinelli, Cattedra di Malattie Metaboliche e del Ricambio, Presidio Ospedaliero di Cisanello Diabetologia, Pisa, Italy; G. Ghirlanda, P. Cotroneo, A. Manto, A. Minella, C. Saponara, Università Cattolica del Sacro Cuore, Rome, Italy; J. Ward, M. Plater, S. Ibrahim, S. Ibbotson, C. Mody, Royal Hallamshire Hospital, Sheffield, U.K. N. Papazoglou, C. Manes, K. Soulis, M. Voukias, Agios Pavlos General Hospital, Thessaloniki, Greece. M. Muggeo, V. Cacciatori, M.L. Gemma, A. Dellera, A. Castellarin, Ospedale Civile, Verona, Italy; K. Irsigler, H. Abrahamian, C. Gurdet, C. Willinger, Hospital Vienna Lainz, Vienna, Austria; A. Nelstrop, C. Feben, Watford General Hospital, Watford, U.K.; S. Walford, V. McLelland, S. Hughes, New Cross Hospital, Wolverhampton, U.K.; Z. Metelko, G. Roglic, Z. Resman, Vuk Vrhovak Institute for Diabetes, Zagreb, Croatia.

Steering committee members: B. Ferriss, Chairman (Cork), J.H. Fuller (London), B. Karamanos (Athens), Z. Kerenyi (Budapest), G. Michel (Luxembourg), M. Muggeo (Verona), J. Stephenson (London), N. Chaturvedi (London), G.C. Viberti (London), A.K. Sjolie (Aarhus).

Coordinating center: J.H. Fuller, N. Chaturvedi, J.M. Stephenson, J. Holloway, M. Milne, D. Webb (London).

Safety committee members: C. Bulpitt, Hammersmith Hospital; A. Fletcher, London School of Hygiene and Tropical Medicine; M. Shipley, University College London.

Central laboratory: G. John, D. Newman, Royal London Hospital.

ACE gene analysis: Phillipa Talmud, Rayne Institute, University College London.

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