Prognostic Effect of Insertion/Deletion Polymorphism of the ACE Gene on Renal and Cardiovascular Clinical Outcomes in Chinese Patients With Type 2 Diabetes

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OBJECTIVE — The insertion/deletion (I/D) polymorphism of the ACE gene has been reported to be associated with diabetic microvascular or macrovascular complications. The aim of the present study was to investigate the prognostic effect of I/D polymorphism on renal and cardiovascular clinical outcomes in Chinese patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — A consecutive cohort of 1,281 Chinese patients with type 2 diabetes were followed for 41.3 ± 21.6 months. Renal end points were defined as renal death and events (need for dialysis, plasma creatinine ≥500 μmol/l, or doubling of plasma creatinine of baseline value ≥150 μmol/l). Cardiovascular end points were defined as cardiovascular death and events, which included ischemic heart disease, heart failure, cerebrovascular accident, and revascularization requiring hospital admission. The I/D polymorphism of the ACE gene was examined by PCR followed by agarose gel electrophoresis.

RESULTS — The frequencies of ACE gene I/D polymorphisms were in Hardy-Weinberg equilibrium. Patients who developed a renal end point (n = 98) had higher frequencies of DD genotype (19.4 vs. 10.8%, P = 0.018) and D allele (41.3 vs. 31.8%, P = 0.006) compared with subjects who did not (n = 1,183). The cumulative rates of renal end points were 10.0, 19.2, and 24.4% in the II (n = 595), DI (n = 539), and DD genotype carriers (n = 147), respectively (log rank P = 0.004). In multiple Cox regression analysis, the occurrence of renal end points remained significantly influenced by I/D polymorphism with a dominant deleterious effect of the DD genotype (DD versus II, adjusted hazard ratio 2.80 [95% CI 1.49–5.29]). There was no prognostic effect of I/D polymorphism on cardiovascular end points.

CONCLUSIONS — The DD genotype of the ACE I/D polymorphism was an independent risk factor for renal but not cardiovascular end points in Chinese patients with type 2 diabetes.

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ACE is one of the key enzymes in the renin-angiotensin system, which plays an important role in fluid and electrolyte balance and regulation of blood pressure and cellular growth (1,2). The human ACE gene is located in chromosome 17q23 with 26 exons and 25 introns with an insertion/deletion (I/D) polymorphism that comprises a 278-bp fragment in intron 16 (3). The DD genotype or D allele of this polymorphism was shown to be associated with elevated circulating and tissue ACE activity (4) as well as increased risk of hypertension (5) and diabetic renal (6) and cardiovascular complications (7). However, these results remained inconsistent in both Caucasian (8–11) and non-Caucasian populations, including Chinese (12,13). These discrepancies might be due to ethnicity, study design, patient selection criteria, and small sample size. The aim of the present study was to evaluate the prognostic effect of this polymorphism on renal and cardiovascular outcomes in a large cohort of 1,281 Chinese patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The Prince of Wales Hospital is the teaching hospital of the Chinese University of Hong Kong. It serves a population of over 1.2 million. Since 1995, as part of a continuous quality improvement program, all newly referred patients to the clinic underwent a comprehensive assessment of complications and risk factors based on the European DiabCare protocol (14). Clinical assessments included measurement of BMI, waist-to-hip ratio, and blood pressure as well as documentation of visual acuity and examination by funduscopy through dilated pupils. For the foot examination, we used monofilament and graduated tuning forks to assess sensory neuropathy. Fasting blood samples were taken for measurement of plasma glucose, HbA_{1c}, lipids (total cholesterol), HDL cholesterol, triglycerides, and plasma creatinine. A sterile, random spot urine sample was used to measure albumin-to-creatinine ratio (ACR) followed by a timed collection (4 or 24 h) for albumin excretion rate (AER). Using the ACR from these two samples, normoalbuminuria was defined as a mean ACR ≤3.5 mg/

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Abbreviations: ACR, albumin-to-creatinine ratio; AER, albumin excretion rate; I/D, insertion/deletion.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Baseline clinical and biochemical characteristics of Chinese patients with type 2 diabetes divided according to their ACE I/D genotype*

	II	DI	DD	*P*
n	595	539	147	
Sex (% women)	44.5	41.5	36.2	0.156
Age (years)	61.0 ± 12.2	60.9 ± 12.2	61.3 ± 10.9	0.928
Duration of diabetes (years)	8.4 ± 6.5	8.4 ± 6.6	8.6 ± 6.5	0.907
BMI (kg/m^2)	24.7 ± 3.7	24.7 ± 3.9	25.0 ± 3.6	0.705
Waist-to-hip ratio	0.89 ± 0.06	0.89 ± 0.09	0.88 ± 0.06	0.778
Systolic blood pressure (mmHg)	138 ± 23	137 ± 23	139 ± 22	0.561
Diastolic blood pressure (mmHg)	79 ± 11	79 ± 11	80 ± 11	0.632
HbA_{1c} (%)	7.7 (6.7–9.2)	7.5 (6.6–8.8)	7.4 (6.7–8.5)	0.581
Fasting plasma glucose (mmol/l)	8.0 (6.5–10.5)	8.0 (6.4–10.5)	8.4 (6.5–10.7)	0.811
Total cholesterol (mmol/l)	5.5 ± 1.2	5.6 ± 1.3	5.6 ± 1.2	0.454
Triglycerides (mmol/l)	1.38 (0.95–2.06)	1.37 (0.88–2.10)	1.35 (0.94–2.12)	0.941
HDL cholesterol (mmol/l)	1.20 (1.01–1.46)	1.23 (1.01–1.53)	1.22 (1.03–1.39)	0.347
LDL cholesterol (mmol/l)	3.4 ± 1.0	3.5 ± 1.1	3.5 ± 0.9	0.470
Plasma creatinine (μmol/l)	75 (61–96)	75 (61–97)	78 (64–99)	0.099
Urinary ACR (mg/mmol)	2.1 (0.7–37.7)	1.7 (0.6–34.6)	2.0 (0.8–35.3)	0.973
Urinary AER (μg/min)	21.3 (8.8–319.0)	20.2 (8.8–489.0)	16.7 (9.8–450.0)	0.784
Serum ACE activity (units/l)*	46.3 ± 38.6	56.8 ± 21.3	63.9 ± 21.9	<0.001
Use of lipid-lowering drugs (%)	32.4	31.5	35.5	0.226
Use of antihypertensive drugs (%)	24.0	24.7	29.6	0.652
Ophthalmic complications (%)†	38.2	36.0	32.2	0.355
Sensory neuropathy (%)	24.0	23.5	28.9	0.518
Cardiovascular complications (%)‡	14.7	13.7	18.4	0.346

Data are means ± SD or median (interquartile range). *P* value is the significance of ANCOVA or χ^2 test. *Serum ACE activity was available in 399 patients. †Ophthalmic complications were defined as diabetic retinopathy, glaucoma, or cataract with impaired visual acuity <20/70 in the same eye. ‡Cardiovascular complications were defined as ischemic heart disease, cerebrovascular disease, and/or peripheral vascular disease.

mmol; microalbuminuria was defined as ACR between 3.5 and 25 mg/mmol; and macroalbuminuria was defined as ACR ≥25 mg/mmol (15). The procedures related to the study were approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong. Informed consent was obtained from all participants.

Between July 1994 and June 1998, a consecutive cohort of 1,281 Chinese patients with type 2 diabetes underwent detailed assessments using the above protocol. Patients with type 1 presentation, defined as diabetic ketoacidosis, acute presentation with heavy ketonuria (>3+), or continuous requirement of insulin within 1 year of diagnosis (16), were excluded. Mortality and clinical outcomes were ascertained in May 2001. Mortality data obtained from the Hong Kong Death Registry were further ascertained by review of case notes. Details of all medical admissions with primary and secondary diagnosis as well as medication history and last available plasma creatinine results were retrieved from the Central Computerized System at the Hospital Authority Head Office, which captures >90% of these data from all public hospitals in Hong Kong. Cardiovascular end points were defined as hospitalizations due to ischemic heart disease, heart failure, cerebrovascular accident, and revascularization procedures. Renal end points were defined as dialysis or doubling of plasma creatinine if the baseline value was ≥150 μmol/l or the absolute value was ≥500 μmol/l.

Laboratory assays

Plasma glucose was measured by a hexokinase method (Hitachi 911 automated analyzer; Boehringer Mannheim, Mannheim, Germany). HbA_{1c} was measured by an automated ion-exchange chromatographic method (reference range 5.1–6.4%; Bio-Rad, Hercules, CA). Interassay and intra-assay coefficient of variation (CV) for HbA_{1c} was ≤3.1% at values <6.5%. Total cholesterol, triglycerides, and HDL cholesterol were measured by enzymatic methods on a Hitachi 911 automated analyzer using reagent kits supplied by the manufacturer of the analyzer. LDL cholesterol was calculated by the Friedewald's equation for triglyceride levels <4.5 mmol/l (17). The precision performance of these assays was within the manufacturer's specifications. Urinary creatinine (Jaffe's kinetic method) and albumin (immunoturbidimetry method) were also measured on the Hitachi 911 analyzer using reagent kits supplied by the manufacturer. The interassay precision CV was 12.0 and 2.3% for urinary albumin concentrations of 8.0 mg/l and 68.8 mg/l, respectively. The lowest detection limit was 3.0 mg/l. Serum creatinine (Jaffe's kinetic method) was measured on a Dimension AR system (Dade Behring, Deerfield, IL). Serum ACE activity was measured by a modified spectrophotometric method with interassay and intra-assay CV <5% (18).

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes. Genotyping for the ACE gene I/D polymorphism was performed using a PCR method as described previously (19). PCR amplification showed a 490-bp product (I allele) and/or 190-bp product (D allele) depending on the presence or absence of the insertion of a 278-bp fragment.

Table 2—*Genotype and allele frequencies of ACE I/D polymorphism in 1,281 Chinese patients with type 2 diabetes with or without occurrence of renal and cardiovascular end points*

	Status of renal end point		Status of cardiovascular end point	
	Nonoccurrence	Occurrence	Nonoccurrence	Occurrence
Genotype frequency				
II	559 (47.3)	36 (36.7)	524 (45.9)	71 (51.1)
DI	496 (41.9)	43 (43.9)	492 (43.1)	47 (33.8)
DD	128 (10.8)	19 (19.4)*	126 (11.0)	21 (15.1)
Total number of genotypes	1,183	98	1,142	139
Allele frequency				
I	1,614 (68.2)	115 (58.7)	1,540 (67.4)	189 (68.0)
D	752 (31.8)	81 (41.3)†	744 (32.6)	89 (32.0)
Total number of alleles	2,366	196	2,284	278

Data are *n* (%). Genotype and allele frequencies were compared by χ^2 test between patients with and without renal and cardiovascular end points. *$P = 0.018$, †$P = 0.006$ when the genotype and allele frequencies were compared between patients with and those without occurrence of renal end points, respectively.

Statistical analysis

SPSS statistical software (version 9.0; SPSS, Chicago, IL) was used for statistical analysis with logarithmic transformation of skewed data including HbA_{1c}, fasting blood glucose, triglycerides, serum creatinine, ACR, and AER. Continuous variables are expressed as means ± SD or median (interquartile range) where appropriate. Between-group comparisons were analyzed using independent Student's *t* test and ANCOVA. The χ^2 test was used to analyze allele and genotype frequencies as well as the frequencies of diabetes complications. Cox regression model was used to estimate the hazard ratio (HR) with 95% CIs for mortality and clinical end points. Kaplan-Meier analysis was used to estimate the cumulative incidence of death and cardiovascular and renal outcomes. A *P* value <0.05 (two-tailed) was considered significant.

RESULTS — A total of 1,281 (41.6% men, mean age 61.0 ± 12.0 years) patients with mean follow-up duration of 41.3 ± 21.6 months were enrolled for the analysis. The frequencies of normoalbuminuria, microalbuminuria, and macroalbuminuria in this prospective cohort were 56.7, 16.1, and 27.2%, respectively. Of these, 98 patients developed renal end points, whereas 139 patients developed cardiovascular end points. Patients who developed renal end points were older (65.2 ± 11.1 vs. 60.6 ± 12.1 years, $P < 0.001$), had longer duration of diabetes (9.6 ± 5.2 vs. 8.3 ± 6.7 years, $P = 0.025$), and were more likely to be men (52.9 vs. 41.4%, $P = 0.03$). They also had higher systolic blood pressure (158 ± 26

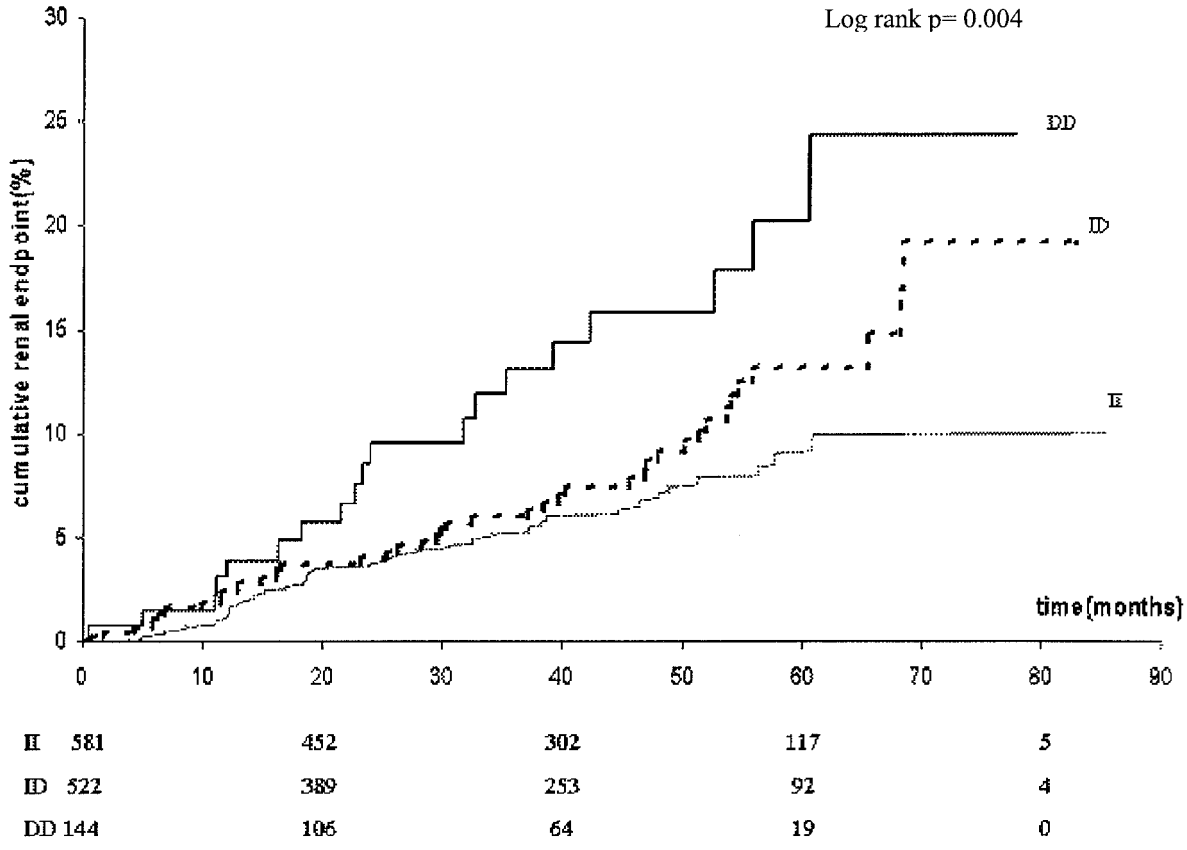

Figure 1—*Cumulative renal end points of 1,281 Chinese patients with type 2 diabetes categorized according to their ACE polymorphism genotypes.*

Table 3—*Multiple Cox regression analysis to examine the predictors for renal and cardiovascular end points in 1,281 Chinese patients with type 2 diabetes*

Independent variables (at baseline)	HR	95% CI	*P*
Renal end point			
First model (without inclusion of serum ACE activity)			
Systolic blood pressure (mmHg)	1.02	1.01–1.03	<0.001
Ln value of triglycerides (mmol/l)	1.38	1.08–1.76	0.0098
Presence of diabetes complications at baseline*	6.99	4.41–11.07	<0.001
ACE genotype			0.004
DI genotype carriers of ACE gene I/D†	1.61	1.01–2.58	0.04
DD genotype carriers of ACE gene I/D‡	2.80	1.49–5.29	0.001
Second model (with serum ACE activity)			
Duration of diabetes (years)	1.07	1.02–1.12	0.005
Ln value of triglycerides (mmol/l)	2.65	1.72–4.07	<0.001
Presence of diabetes complications at baseline*	5.60	2.62–11.98	<0.001
ACE genotype			0.18
DI genotype carriers of ACE gene I/D†	2.51	0.84–7.49	0.19
DD genotype carriers of ACE gene I/D‡	2.46	0.36–16.55	0.12
Cardiovascular end points			
Age	1.10	1.06–1.09	<0.001
Systolic blood pressure (mmHg)	1.02	1.01–1.03	<0.001
LDL cholesterol (mmol/l)	1.24	1.05–1.45	0.009
Presence of diabetes complications at baseline*	2.12	1.43–3.14	0.0002

Dependent variable: renal end point defined as dialysis or doubling of baseline plasma creatinine or absolute value ≥500 μmol/l. Independent variables for renal end points: age, duration of diabetes, systolic blood pressure, diastolic blood pressure, total cholesterol, ln value of triglycerides, presence of diabetes complications at baseline, and ACE gene I/D polymorphism II, DI, and DD genotype. Independent variables for cardiovascular end point: age, duration of diabetes, systolic blood pressure, diastolic blood pressure, ln value of triglycerides, LDL cholesterol, presence of diabetes complications at baseline, and ACE gene I/D polymorphism II, DI, and DD genotype. *Presence of diabetes complications at baseline, including cardiovascular complications (ischemic heart disease, heart failure, stroke and/or peripheral vascular disease) or renal impairment with plasma creatinine ≥150 μmol/l or presence of retinopathy. †DI genotype carriers compared with II genotype carriers. ‡DD genotype carriers compared with II genotype carriers.

vs. 136 ± 22 mmHg, $P < 0.001$), greater waist-to-hip ratio (0.90 ± 0.07 vs. 0.89 ± 0.07, $P < 0.001$), more adverse lipid profile (total cholesterol 6.2 ± 1.6 vs. 5.5 ± 1.2 mmol/l, $P < 0.001$; triglycerides: median 1.81 [interquartile range 1.25–2.74] vs. 1.35 mmol/l [0.91–2.02], $P < 0.001$), less glycemic control (HbA_{1c} 8.1 ± 2.2 vs. 7.9 ± 1.9%, $P < 0.001$), higher serum creatinine (199 [150–330] vs. 73 μmol/l [60–91], $P < 0.001$), and AER (2,567.4 [1,534.5–4,139.1] vs. 16.7 μg/min [8.5–154.0], $P < 0.001$) after adjustment for sex, age, and duration of diabetes. Patients who developed renal end points were also more likely to have retinopathy (77.9 vs. 33.1%, $P < 0.001$) and cardiovascular disease (25.0 vs. 3.8%, $P = 0.003$) at baseline and higher use of antihypertensive (66.3 vs. 29.2%, $P < 0.001$) and lipid-lowering drugs (56.7 vs. 30.3%, $P < 0.001$). Similarly, patients who developed cardiovascular end points had worse clinical profiles than patients who did not (data not shown). In this cohort, a random sample of 399 patients underwent measurement of serum ACE activity. They had similar clinical characteristics as those in whom serum ACE activity was not available (data not shown).

The frequency was 67.5% for the I allele and 32.5% for the D allele; observed genotype frequencies were 46.4, 42.1, and 11.5% for the II, DI, and DD alleles, respectively. Genotype frequencies were in Hardy-Weinberg equilibrium. Table 1 summarizes the clinical and biochemical profiles of patients according to genotypes. They had comparable age, duration of diabetes, and anthropometric measurements, except that D allele carriers tended to have more cardiovascular complications and were more likely to be treated with antihypertensive and lipid-lowering drugs. In a subgroup of patients in whom serum ACE activity was available, DD ($n = 40$) carriers had higher serum ACE activity than II ($n = 193$) and DI ($n = 166$) carriers (DD vs. DI vs. II, 63.9 ± 21.9 vs. 56.8 ± 21.3 vs. 46.3 ± 38.6 units/l, $P < 0.001$). There was a weak but significant correlation between AER and serum ACE activity ($r = 0.12$, $P = 0.003$).

Patients who developed renal end points had higher proportions of the DD genotype (19.4 vs. 10.8%, $P = 0.018$) and D allele carriers (41.3 vs. 31.8%, $P = 0.006$) than patients who did not (Table 2). The cumulative rates of renal end points were 10.0, 19.2, and 24.4% in II, DI, and DD carriers, respectively (Fig. 1). Using Cox regression analysis, ACE I/D polymorphism was an independent predictor for development of renal end points after controlling all confounding factors. Compared with patients with II genotype, DD genotype conferred an approximately threefold increased risk (HR 2.80 [95% CI 1.49–5.29], $P = 0.001$), whereas DI genotype, a 1.6-fold increased risk (HR 1.61 [1.01–2.58], $P = 0.04$) for renal end points. Other independent predictors included blood pressure, triglycerides, and underlying cardiovascular complications, renal impairment defined as plasma creatinine ≥150 μmol/l, or presence of retinopathy at baseline. The conferred risk by DD/DI genotypes on renal end points became statistically insignificant when serum ACE activity was included in the model, whereas ACE activity per se was not an independent factor for renal events (HR 1.00 [0.98–1.02]) (Table 3).

Using Kaplan-Meier analysis, there was also a trend for DD carriers to develop more cardiovascular end points than II carriers (HR 1.27 [0.77–2.08]) but this did not reach statistical significance (Table 2 and Fig. 2). In the Cox regression analysis, age, blood pressure, LDL cholesterol, and presence of diabetes complications at baseline were independent risk factors for development of cardiovascular end points (Table 3).

CONCLUSIONS — In this prospective cohort of 1,281 Chinese patients with type 2 diabetes, we had demonstrated the deleterious effects of the D allele of the ACE I/D polymorphism on progression of nephropathy in addition to other risk factors such as blood pressure and lipid control as well as presence of diabetes complications. This is in accordance with recent findings that D allele was associated with development of nephropathy and structural kidney damage (9,20).

Albuminuria, blood pressure, and metabolic control are important promot-

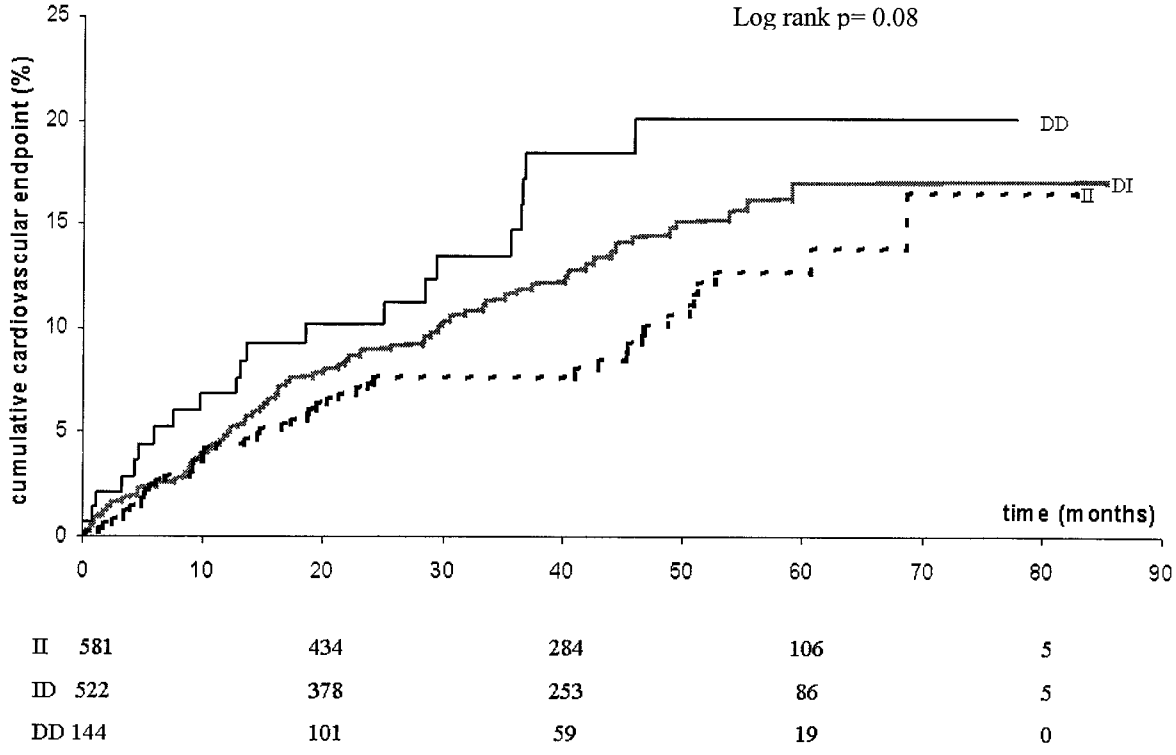

Figure 2—*Cumulative cardiovascular end points of 1,281 Chinese patients with type 2 diabetes categorized according to their ACE polymorphism genotypes.*

ers of diabetic nephropathy, but these factors only accounted for approximately one-third of the variability (21). On the other hand, genetic factors had been shown to modulate risk of development of nephropathy in family studies (22). The ACE I/D polymorphism is one of the most extensively studied candidate genes and accounts for >40% of interindividual variability of serum or tissue ACE activity (23). In this respect, ACE activity is important in determining intrarenal angiotensin and kinin levels, which in turn control intraglomerular pressure and development of kidney damage via increased angiotensin II and probably reduced kinin formation (24,25). Increased serum ACE activity has been reported in both type 1 and type 2 diabetic patients with microalbuminuria (26,27). In addition, both animal and clinical studies have shown that ACE inhibition reduced intraglomerular pressure and attenuated the progression of nephropathy (28,29). In this study, patients with renal end points had more adverse metabolic profile, cardiovascular risk factors, renal impairment, albuminuria, and retinopathy at baseline. However, many of these associations were not selected as significant independent variables in the multivariate model, possibly due to the overwhelming effect of cardiovascular complications that are known to be closely associated with multiple risk factors. Nevertheless, systolic blood pressure and log value of triglycerides remained significant predictors for renal end points after controlling for all these confounding variables. Notably, every 1-mmHg increment of systolic blood pressure and 1 log value of triglycerides was associated with a 1.02- and 1.38-fold increased risk, respectively. The results illustrated the importance of an integrated approach involving control of multiple risk factors in these high-risk patients.

On the other hand, after controlling for these confounding factors, the DD genotype remained an independent risk factor for renal end points, with an HR of 2.8. However, when serum ACE activity was included in the model, the independent predictive value of DD genotype lost its significance. In this connection, in keeping with other studies, we observed a small but significant association between serum ACE activity and AER, suggesting that the prognostic effect of ACE I/D polymorphism might be mediated, in part, by ACE activity or possibly other closely associated factors that were not measured in this study.

In this cohort of 1,281 patients, there were 139 cardiovascular and 98 renal end points during a mean follow-up period of 41 months. These findings highlight the equally important roles of cardiovascular and renal events in Chinese compared with Caucasians, in whom cardiovascular morbidity and mortality predominate (30). It is now established that Caucasians have higher frequencies of the DD genotype, ranging from 32 to 42%, compared with 14–18% as reported in Asians (31–34). In our study, the prevalence of the DD genotype was 11.5%. Although these interethnic differences in genotype distribution have been put forward by some workers to explain the low prevalence of cardiovascular disease in Asians, the roles of ACE I/D polymorphism in hypertension and cardiovascular disease in Asians remain controversial (31,35–37). In this prospective cohort analysis, we observed a tendency for increased cardiovascular end points among DD genotype carriers, albeit short of significance.

Several potential limitations of this large-scale observational study warrant

further discussion. First, proteinuria and progression of nephropathy may be confounded by other factors, including changes in blood pressure, obesity, dyslipidemia, and glycemic control throughout the study period. In addition, adjustment of types and dosages of antihypertensive treatments, particularly ACE inhibitors, might confound the results given the weak but significant associations between AER and serum ACE activity. On the other hand, a previous study (38) suggested that serum ACE activity was an independent risk factor for development of cardiovascular end points along with other conventional risk factors. In our study, ACE activity was only measured in a small proportion of our patients, and further studies are required to address this issue. In this respect, survival bias due to premature death from cardiovascular disease among D allele carriers remains a possibility (8). Nevertheless, genotype distribution in our cohort was in Hardy-Weinberg equilibrium and the association between cardiovascular outcome and ACE I/D polymorphism was insignificant, suggesting minimal dropout. Despite these limitations, which would tend to weaken our results, we were able to demonstrate the prognostic significance of ACE I/D polymorphism, in addition to other conventional risk factors, in the progression of renal function in Chinese patients with type 2 diabetes.

In conclusion, in Chinese type 2 diabetic patients, the ACE D allele was associated with an increased risk of progression of nephropathy, which was mediated, in part, through its effect on serum ACE activity.

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