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Angiotensin-converting enzyme (ACE) inhibition in type 2, diabetic patients – interaction with ACE insertion/deletion polymorphism

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Angiotensin-converting enzyme (ACE) insertion(I)/deletion (D) polymorphism may modify the effect of inhibition of the renin–angiotensin–aldosterone system (RAAS) on survival and cardiorenal outcomes in type 2, diabetes. A consecutive cohort of 2089 Chinese type 2 diabetic patients with mean (±standard deviation) age of 59.7±13.1 years were genotyped for this polymorphism by polymerase chain reaction method and were followed prospectively for a median period of 44.6 (interquartile range: 23.7, 57.5) months. Clinical outcomes, including all-cause mortality, cardiovascular and renal end points, were examined. The frequency for I allele was 67.1 and 32.9% for D allele, with observed genotype frequencies of 45.8, 42.6, and 11.6% for 3, DI and DD, respectively. ACE DD polymorphism was an independent predictor for renal end point with hazard ratio (HR) (95% confidence interval) of 1.72 (1.16, 2.56), but not for cardiovascular end point or mortality. After controlling for confounding factors, including ACE I/D genotype, the usage of RAAS inhibitors was associated with reduced risk of mortality (HR 0.34 (0.23, 0.50)) and renal end point (HR 0.55 (0.40, 0.75)). On subgroup analysis, the beneficial effects on survival (II vs DI vs DD: HR 0.29 (0.16, 0.51) vs 0.25 (0.14, 0.46) vs 1.33 (0.41, 4.31)) and renoprotection (II vs DI vs DD: 0.52 (0.30, 0.90) vs 0.43 (0.25, 0.72) vs 0.95 (0.43, 2.12)) were most evident in II and DI carriers. In conclusion, inhibition of RAAS was associated with reduced risk of mortality and occurrence of renal end point in Chinese type 2 diabetic patients. These benefits were most evident among II and DI carriers.

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Angiotensin-converting enzyme (ACE) is one of the key enzymes in the renin–angiotensin–aldosterone system (RAAS). The insertion (I)/deletion (D) polymorphism of this gene has been studied extensively with respect to its association with diabetic renal[1] and cardiovascular complications.[2] Patients with DD genotype or D allele have elevated circulating and tissue ACE activity[3] compared to patients with I allele. This may contribute to the interindividual variability in the antiproteinuric responses to inhibition of the RAAS using either ACE inhibitor or AII receptor blockers (ARB).[4] Although the beneficial effects of RAAS inhibition on cardiorenal end points in type 2 diabetic patients have been confirmed by several landmark studies,[5–8] examination of pharmacogenetics in diabetic nephropathy may improve our understanding of the heterogeneity of treatment responses and help identify groups of patients who are most likely to benefit from the drug as well as develop alternative hypothesis among nonresponders. In this large-scale observational study of Chinese type 2 diabetic patients, we examined the effects of RAAS inhibition on survival and cardiorenal outcomes in relation to ACE I/D polymorphism in a consecutive cohort referred to our clinic since 1995.

RESULTS

A total of 2089 (41.4% males, mean age 59.7±13.1 years) patients with median follow-up of 44.6 (23.7, 57.5) months were enrolled for survival analysis. The frequencies of normo-, micro- and macroalbuminuria in this prospective cohort were 58.6, 21.2 and 20.2%, respectively. The frequency for I allele was 67.1 and 32.9% for D allele, with observed genotype frequencies of 45.8, 42.6 and 11.6% for II, DI and DD, 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 clinical characteristics, including the albuminuric status. Serum ACE activity was measured in a random cohort of 718 patients. In this subgroup, DD carriers ($n=75$) had higher serum ACE activity than DI ($n=310$) and II carriers ($n=333$) (61.8±20.5 vs 56.6±23.0 vs 44.5±31.2 U/l, $P<0.001$). These

Table 1 | Baseline characteristics of 2089 Chinese type 2 diabetic patients categorized according to their ACE I/D polymorphism genotypes

	II (n=956)	ID (n=890)	DD (n=243)	P-value
Age (year)	59.9±12.7	59.1±13.4	60.7±13.0	0.19
Male (%)	43.2	39.9	39.5	0.29
Duration of diabetes (year)	7.1±6.3	7.0±6.4	7.1±6.3	0.92
Family history of diabetes	39.6	38.2	39.1	0.82
Smoking (% current/ex-smoker)	28.9	28.9	27.2	0.85
Systolic BP (mmHg)	137±22	135±24	136±21	0.19
Diastolic BP (mmHg)	80±11	79±12	80±11	0.12
Waist to hip ratio	0.89±0.07	0.88±0.08	0.89±0.06	0.56
Body mass index (kg/m^2)	24.9±3.8	24.9±3.9	24.8±3.6	0.97
HbA_{1c} (%)	7.9±1.9	7.8±1.9	7.9±2.0	0.81
Fasting plasma glucose (mmol/l)	8.9±3.7	8.8±3.4	9.2±3.5	0.25
Total cholesterol (mmol/l)	5.5±1.2	5.5±1.3	5.5±1.3	0.69
Triglyceride (mmol/l)	1.39 (0.96, 2.07)	1.36 (0.89, 2.00)	1.32 (0.94, 2.02)	0.43
HDL-C (mmol/l)	1.2±0.3	1.3±0.4	1.3±0.4	0.21
LDL-C (mmol/l)	3.4±0.9	3.5±1.1	3.5±0.9	0.31
Plasma creatinine (μmol/l)	73 (60, 91)	72 (60, 91)	74 (63, 93)	0.30
Estimated GFR (ml/min/1.73 m^2)	89.5 (70.6, 109.6)	90.2 (72.1, 107.3)	85.3 (68.5, 101.8)	0.08
4-h albumin excretion rate (μg/min)	19.2 (8.7, 113.0)	18.6 (8.4, 132.1)	16.7 (9.2, 138.4)	0.98
Spot urine albumin:creatinine ratio (mg/mmol)	1.87 (0.68, 13.48)	1.57 (0.60, 10.40)	1.85 (0.73, 15.58)	0.64
Serum ACE activity (U/l)[a]	44.5±31.2	56.6±23.0	61.8±20.5	<0.001
Renal insufficiency (%)[b]	15.7	17.9	18.2	0.40
Albuminuric status (%)				0.59
Normoalbuminuric	57.4	59.1	61.8	
Microalbuminuric	22.4	20.8	17.6	
Macroalbuminuric	20.2	20.0	20.6	
Use of antihypertensive treatment (%)	30.1	29.5	34.8	0.56
Use of lipid-lowering treatment (%)	30.2	29.9	32.9	0.62
Presence of cardiovascular complications (%)[c]	12.7	12.6	16.9	0.18
Presence of retinopathy (%)	32.8	30.5	35.0	0.33

Data are presented as mean±s.d. or median (interquartile range) where appropriate.
[a]A random sample of 718 patients had serum ACE activity measured and their clinical characteristics were not statistically different from other patients.
[b]Renal insufficiency was defined as serum creatinine ⩾150 μmol/l and/or eGFR <60 ml/min/1.73 m^2.
[c]Cardiovascular complications were defined as the presence of congestive heart failure, ischemic heart disease, revascularization, cerebrovascular accident or peripheral vascular disease at baseline.

patients had similar clinical characteristics as those in whom serum ACE activity was not available (data not shown).

RAAS inhibitors were used in 45.6, 42.9, and 40.7% of II, DI and DD carriers, respectively. The majority of these patients (84.5%) were treated with an ACE inhibitor. A small proportion (15.5%) of patients had been treated with an ACE inhibitor and then switched to an ARB, or treated with an ARB as initial therapy. None of the patients were on combination therapy with ACE inhibitor and ARB. Compared to ACE inhibitor users, ARB users had higher systolic blood pressure (150±27 vs 144±21 mmHg, $P=0.001$) and higher serum creatinine (106 (77, 151) vs 79 (64, 98) μmol/l, $P<0.001$).

The usage of RAAS inhibitors was significantly associated with reduced mortality after controlling for other confounding factors, including ACE I/D genotype (hazard ratio (HR) 0.34 (0.23, 0.50)). Other predicting factors for mortality included age, duration of diabetes, albumin excretion rate (AER) and presence of macrovascular complications at baseline. Although use of RAAS inhibitor was associated with reduced mortality, ACE I/D genotype was not a predictor for survival (data not shown). On subgroup analysis, the beneficial effect of RAAS inhibition on survival was most evident in II and DI carriers (II vs DI vs DD: HR 0.29 (0.16, 0.51) vs 0.25 (0.14, 0.46) vs 1.33 (0.41, 4.31)).

The cumulative rates of renal end point were 21.6, 34.8 and 36.5% in II, DI and DD carriers, respectively. On multivariate analysis, after controlling for confounding factors and compared to patients with II genotype, DD genotype conferred an approximately two-fold increased risk of renal end point. In the whole group, RAAS inhibition was associated with reduced risk of occurrence of renal end point even controlling for ACE I/D genotype (Table 2, Model 1). On stratifying patients according to their genotypes and usage of RAAS inhibitors, the renoprotective effects of RAAS inhibition were most evident in patients with II and DI genotypes (Table 2, Model 2). Other predictors for renal end point include albuminuria, systolic blood pressure, body mass index, presence of macrovascular, and ophthalmic complications at baseline. As shown in Figure 1, in the subgroup of patients treated with RAAS inhibition, DD carriers had the highest risk of development of composite renal end point (DD carriers vs II carriers OR 1.84 (95% confidence interval (CI): 1.11, 3.05)). Table 3 summarizes the effects of RAAS inhibition on composite renal end points categorized by baseline albuminuric status and genotype of

Table 2 | Multivariate analysis using Cox regression model, showing the HRs (95% CIs) of various predictors for composite renal end point in 2089 Chinese type 2 diabetic patients[a]

Variable	HR (95% CI)	*P*-value
Model 1[b]		
Ln 4-h albumin excretion rate (μg/min)	1.92 (1.77, 2.09)	<0.001
Systolic blood pressure (mmHg)	1.01 (1.00, 1.02)	0.006
Body mass index (kg/m^2)	0.92 (0.89, 0.97)	0.001
Presence of macrovascular complications	1.77 (1.27, 2.45)	0.0007
Presence of ophthalmic complications	2.33 (1.64, 3.30)	<0.001
ACE I/D polymorphism[c]		
DI carriers	1.20 (0.89, 1.62)	0.22
DD carriers	1.72 (1.16, 2.56)	0.007
Usage of RAAS inhibition	0.55 (0.40, 0.75)	0.0002
Model 2		
Ln 4-h albumin excretion rate (μg/min)	1.92 (1.77, 2.09)	<0.001
Systolic blood pressure (mmHg)	1.01 (1.00, 1.02)	0.006
Body mass index (kg/m^2)	0.92 (0.89, 0.97)	0.001
Presence of macrovascular complications	1.77 (1.27, 2.45)	0.0007
Presence of ophthalmic complications	2.33 (1.64, 3.30)	<0.001
ACE I/D polymorphism		0.03
Usage of RAAS inhibition		
Usage of RAAS inhibitor and II genotype	0.52 (0.30, 0.90)	0.02
Usage of RAAS inhibitor and DI genotype	0.43 (0.25, 0.72)	0.001
Usage of RAAS inhibitor and DD genotype	0.95 (0.43, 2.12)	0.91

Other independent variables, including age, male sex, duration of diabetes, baseline HbA_{1c} and total cholesterol, were not selected in the model.
[a]Time to event analyses using Cox regression to calculate the risk of occurrence of renal end point expressed as HR with 95% CI. Renal end point was defined as death due to renal failure, dialysis, eGFR <15 ml/min/1.73m^2 or more than 50% loss of eGFR compared with baseline using the Modification of Diet in Renal Disease equation.
[b]Model 1 estimated the risk association of ACE I/D polymorphism and renal end point after adjustment for other confounding factors, including usage of RAAS inhibition. Model 2 estimated the renoprotective effect of RAAS inhibition with respect to ACE I/D polymorphism after adjustment for other confounding factors.
[c]In estimating the risk association of ACE I/D polymorphism and renal end point, II genotype was used as the reference group for comparison with the DI and DD genotype groups.

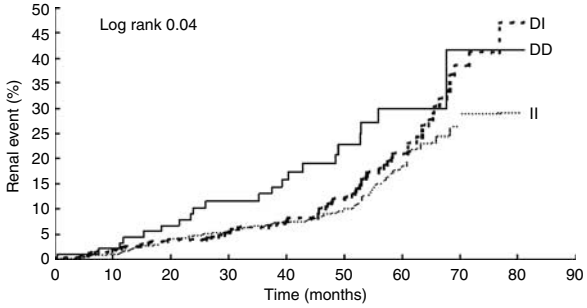

Figure 1 | Cumulative renal events in Chinese type 2 diabetic patients categorized according to ACE I/D genotype, who were treated with RAAS inhibitors ($n = 917$).

ACE I/D polymorphism after adjustment of other confounding factors. The renoprotective effect of RAAS inhibitors was observed in all patients with macroalbuminuria irrespective of their ACE I/D genotype, with the effects most evident in the II carriers.

Table 3 | The impact of renin–angiotensin–aldosterone-system inhibition on risk of occurrence of renal end point in 2089 Chinese type 2 diabetic patients stratified by their ACE I/D genotype and baseline albuminuric status

	II	DI	DD
Macroalbuminuric	0.25 (0.15, 0.42)	0.31 (0.18, 0.53)	0.40 (0.17, 0.94)
Microalbuminuric	1.40 (0.39, 4.97)	1.15 (0.38, 3.51)	1.95 (0.22, 17.48)
Normoalbuminuric	NA	1.99 (0.44, 9.03)	1.26 (0.23, 6.92)

Time to event analyses using Cox regression were performed to calculate the risk of renal events stratified by both ACE I/D polymorphism and baseline albuminuric status, expressed as HR (95% CI).
Renal end point was defined as death due to renal failure, dialysis, eGFR of <15 ml/min/1.73 m^2 or more than 50% loss of eGFR compared with baseline using the Modification of Diet in Renal Disease equation.

There was a trend for DD carriers to develop more cardiovascular end points (HR 1.05 (0.69, 1.59)) than II or DI carriers, albeit short of statistical significance. RAAS inhibition showed a nonsignificant benefit in reducing cardiovascular end point in all patients (data not shown).

DISCUSSION

In this relatively large-scale prospective study, we have shown that both ACE I/D polymorphism and RAAS inhibition were independent predictors for renal end points in Chinese type 2 diabetic patients. Somewhat counter-intuitively but in agreement with previous studies,[9] the benefit of RAAS inhibition on reducing the risk of mortality and renal end point was most evident among II and DI carriers.

Diabetic nephropathy is a major cause of morbidity and mortality, especially in non-Caucasian populations.[10] Both clinical and animal studies have suggested that intrarenal angiotensin II (Ang II) plays a pivotal role in the development of nephropathy. In this connection, blocking of RAAS by ACE inhibitors or ARB has been shown to delay the progression of nephropathy.[5,7] The ACE I/D polymorphism accounts for over 40% of interindividual variability of serum or tissue ACE activity,[11] and its association with diabetic nephropathy has been extensively investigated.[1,12,13] In addition to modifying the metabolic profile, albuminuria, and blood pressure, which collectively account for one-third of the variability of diabetic nephropathy,[14] ACE I/D polymorphism is a potential modulating factor in the progression of nephropathy in both type 1 and type 2 diabetic patients.[15–17]

Apart from having higher serum ACE activity, D allele carriers had similar baseline characteristics as the non-D carriers. However, on prospective analysis, ACE DD genotype was associated with two-fold increased risk of occurrence of renal event, but not for death or cardiovascular events. After controlling for conventional risk factors, including blood pressure, duration of disease, albuminuria, and diabetic complications, RAAS inhibition reduced the risk of occurrence of renal end point by 45% (Table 2) and death by 66%. On subgroup analysis after stratification by ACE D/I genotype and albuminuric status, the renoprotective effects

of RAAS inhibition were most evident in the II and DI carriers (Table 2). Similarly, the effect of RAAS inhibition on mortality was also mainly observed in these two groups. When looked at another way, while RAAS inhibition conferred renoprotection in all patients with macroalbuminuria, the effects were most evident among the II carriers (Table 3). Although these results may be due to the small number of patients with DD genotype, they are consistent with findings from other long-term studies, which also indicated that the antiproteinuric response to ACE inhibition in patients with diabetic nephropathy was attenuated among the DD carriers.[9,18]

Our study cohort was recruited between 1995 and 2000, a time before publication of several landmark studies such as RENAAL, IDNT and LIFE.[6–8] Hence, most of the patients were treated with ACE inhibitors rather than ARB ($n = 323$). In this regard, ACE inhibitor reduces the conversion from Ang I to Ang II, which is accompanied by increased renin and Ang I levels, both of which can result in resistance to ACE inhibition over time.[19] Besides, in both diabetes and hypertension, there is evidence of upregulation of alternative pathways for Ang II production, such as the chymase-mediated activity, which can escape ACE inhibition. In support of this notion, ACE expression within diabetic kidneys does not appear to correlate with degree of glomerulosclerosis and proteinuria.[20] Taken together, it is plausible that combination treatment of ACE inhibitor and ARB may offer more complete blockade of the RAAS and thus reduction of intrarenal Ang II production.[21,22] Indeed, in nondiabetic patients with renal insufficiency, dual blockade of ACE inhibitor and ARB has been shown to be more effective than single therapy of either drug in reducing renal end point.[23] However, similar studies in type 2 diabetes, which is the main contributor to the growing burden of renal disease, are still awaited.

Subjects in our study were not randomized with regard to ACE inhibition, and this may have resulted in selection bias. Other confounding factors, such as changes in antihypertensive therapy or changes in blood pressure, obesity, dyslipidemia, and glycaemic control during the observational period, can influence the progression of nephropathy. Survival bias with certain genotypes may be confounding due to possible association between D allele and premature death from cardiovascular events.[13] However, the genotype distribution in our cohort was in Hardy–Weinberg equilibrium and the association between cardiovascular outcome and ACE I/D polymorphism was insignificant, suggesting that there should be minimal dropout. We were also not able to specifically examine the effect of ARB in different ACE I/D genotype carriers due to the relatively small number of subjects treated with ARB.

In conclusion, we have observed the potential interaction between ACE I/D polymorphism and RAAS inhibition in Chinese type 2 diabetic patients. After controlling for conventional risk factors, ACE DD genotype remained an independent predictor for occurrence of renal end point. While RAAS inhibition was associated with improved survival and renoprotection, these beneficial effects were most evident among the II and DI carriers. Given the high renal risk of patients with DD genotype and their less favorable response to RAAS inhibition, there is a need to explore other treatment modalities to further reduce risk in these patients.

MATERIALS AND METHODS

Patients and method

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. Between 1995 and 2001, a consecutive cohort of 5205 Chinese type 2 diabetic patients from the hospital underwent detailed assessment using the European DiabCare protocol.[24] We have previously reported the prognostic effects of albuminuria in a subgroup of 3773 patients on renal outcome.[25] Among these patients, 1281 had genotyping for ACE I/D polymorphism, which was found to predict the renal outcome.[26] In this analysis, we have performed additional genotyping to increase the latter cohort size to 2089 subjects recruited consecutively from 1995 to 1998 in whom detailed documentation of drug information was also available. Patients with type 1 diabetes, defined as presentation with diabetic ketoacidosis, acute symptoms with heavy ketonuria ($>3+$) or continuous requirement of insulin within 1 year of diagnosis[27] were excluded.

Apart from documentation of demographic data and clinical assessment of diabetic complications, fasting blood samples were taken for measurement of plasma glucose, glycated hemoglobin (HbA_{1c}), lipid profile (total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG), calculated low-density lipoprotein-cholesterol (LDL-C), renal and liver functions. All patients had at least two urinary collections: a sterile, random spot urine sample was used to measure albumin creatinine ratio (ACR), followed by a timed collection (4 or 24 h) for measurement of ACR and AER. None of the patients had known history of glomerulonephritis, microscopic hematuria or known history of obstructive uropathy, such as renal stone on ultrasound scan. Definition of albuminuria was based on the mean value of ACR from both the timed and spot urinary samples. Normoalbuminuria was defined as a mean ACR $\leqslant 3.5$ mg/mmol, microalbuminuria ACR between 3.5 and 25 mg/mmol and macroalbuminuria $\geqslant 25$ mg/mmol.[28]

In this prospective analysis, mortality data were obtained from the Hong Kong Death Registry and 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. The latter is the governing body of all public hospitals in Hong Kong and captures more than 90% of these data. Cardiovascular end point was defined as hospitalizations due to ischemic heart disease, congestive heart failure, stroke and revascularization procedures. Renal end point was defined as death due to renal failure, dialysis, estimated glomerular filtration rate (eGFR) of less than 15 ml/min/1.73 m^2 and/or more than 50% loss of glomerular filtration rate compared with baseline using the Modification of Diet in Renal Disease equation.[29]

Laboratory assays

Plasma glucose was measured by a hexokinase method (Hitachi 911 automated analyzer, Boerhringer Mannheim, Mannheim, Germany).

HbA_{1c} was measured by an automated ion-exchange chromatographic method (Bio-Rad Laboratory, Hercules, CA, USA with reference range: 5.1–6.4%). Inter- and intra-assay coefficient of variation (CV) for HbA_{1c} was $\leqslant 3.1\%$ at values below 6.5%. TC, TG and HDL-C were measured by enzymatic methods on a Hitachi 911 automated analyzer (Boehringer, Mannheim, Germany) using reagent kits supplied by the manufacturer of the analyzer. LDL-C was calculated by the Friedewald's equation for TG <4.5 mmol/l.[30] 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 by the Hitachi 911 analyzer using reagent kits supplied by the manufacturer. The inter-assay precision CV was 12.0 and 2.3% for urinary albumin concentrations of 8.0 and 68.8 mg/l, respectively. The lowest detection limit was 3.0 mg/l. Plasma creatinine (Jaffe's kinetic method) was measured on a Dimension AR system (Dade Behring, Deerfield, IL, USA).

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes. Genotyping for the *ACE* gene I/D polymorphism was performed using the polymerase chain reaction (PCR) method as described previously.[31] PCR amplification revealed 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.

Data analysis

The analysis was performed using the Statistical Package for Social Sciences (version 9.0) statistical package. Plasma TG, plasma creatinine and albuminuria were logarithmically transformed due to skewed distributions. All data are expressed as mean $\pm$ standard deviation or median (interquartile range), as appropriate. The Student's *t*-test or analysis of variance was used for between-group comparisons for continuous variables and χ^2 test for categorical variables. Cox-regression model was used to estimate the HR with 95% CI for mortality and clinical end points, with the assumption that the effects of the different variables on survival are constant. Independent variables were subjected to univariate analysis and then variables showing statistically significant results were entered as covariates in the multivariate analysis. Kaplan–Meier analysis was used to estimate the cumulative incidence of death and cardiorenal outcomes, and log rank test was used to demonstrate the trend for survival. A *P*-value <0.05 (two-tailed) was considered to be significant.

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