


# The association of ACE gene polymorphism with diabetic kidney disease and renoprotective efficacy of valsartan

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## Abstract

**Introduction:** To investigate the associations between the insertion/deletion (I/D) polymorphisms in the angiotensin converting enzyme (ACE) gene and susceptibility to diabetic kidney disease (DKD); and the efficacy of valsartan in reducing the urine protein in Type 2 diabetes mellitus (T2DM) patients.

**Materials and methods:** We enrolled 128 T2DM patients in this study, including 54 cases with DKD (DKD+) and 74 controls (DKD–). The ACE polymorphism was assayed by polymerase chain reaction (PCR), and the genotype distribution and allele frequency were analyzed. The DKD+ group was subdivided into the DD, ID and II subgroups, based on their genotypes. In addition, patients with DKD received valsartan treatment for 12 weeks. We determined changes in the urinary albumin to creatinine ratio (ACR) and serum creatinine (SCr).

**Results:** The frequencies of the genotypes DD and ID were higher in the DKD+ than in the DKD– group. The frequency of allele D was higher, and of allele I was lower, in the DKD+ than in DKD– group ( $p < 0.05$ ). Following valsartan treatment, albuminuria was significantly decreased in subgroups DD and ID ( $p < 0.05$ ).

**Conclusions:** In T2DM patients, the ACE I/D polymorphism was associated with onset of DKD. Furthermore, the ACE I/D polymorphism influenced the renoprotective response to valsartan: Patients with the DD genotype benefitted the most from this treatment.

## Keywords

Angiotensin converting enzyme, diabetes, diabetes mellitus Type 2, diabetic kidney disease, end stage renal disease, gene polymorphisms, kidney disease, microangiopathy, microvascular complications, valsartan

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## Introduction

Diabetic kidney disease (DKD) is one of the serious microvascular complications of diabetes mellitus (DM). About 20–40% of diabetic patients suffer from DKD, which is the leading cause of end stage renal disease (ESRD).<sup>1</sup>

The renin-angiotensin system (RAS) is involved in most of the pathological processes that lead to DKD. Angiotensin II (Ang II) is the major peptide of RAS, and is formed under the action of the angiotensin converting enzyme (ACE). Ang II shows increased activity under high glucose conditions. This causes hypertrophy of various renal cells and has a pressor effect on arteriolar smooth muscles, thereby resulting in increased vascular pressure.

It also induces inflammation, apoptosis, cell growth, migration and differentiation.<sup>2</sup>

Not all patients with poor glycemic control eventually develop DKD; on the contrary, some DM patients with well-controlled blood glucose can still develop this dis-

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ease. Studies show that there is a strong genetic predisposition to the onset and progression of DKD.<sup>3,4</sup>

The *ACE* gene is composed of 26 exons and 25 introns, with a length of 21 kb. There are > 160 *ACE* gene polymorphisms that are currently known; most of them are single nucleotide polymorphisms. Rigat et al.<sup>5</sup> first reported that the *ACE* gene insertion/deletion (*I/D*) polymorphism is caused by the insertion or deletion of a 287 bp Alu repeat sequence within intron 16, which results in three genotypes. While the presence of the DD genotype was found to be associated with the highest level of ACE, the enzyme levels were reported to be the lowest in the II genotype. Recently, many researchers have demonstrated that the *ACE I/D* polymorphism is related to diabetic microangiopathy, and that the D allele might be a susceptibility factor for patients with DKD.<sup>6–8</sup> The frequency of *ACE* alleles varies within different ethnic groups, which might contribute to the conflicting views on the role of the *ACE* gene *I/D* polymorphism in the development of DKD.<sup>9,10</sup>

With advancements in the field of pharmacogenomics, the *ACE I/D* polymorphism was shown to contribute to the therapeutic effect of the Ang II receptor antagonists (ARBs). Ruggenti et al.<sup>11</sup> report that the DD genotype is associated with a better response to ARB therapy in overt nephropathy of Type 2 diabetes, as compared to the II genotype. A study by Felehgari et al.<sup>10</sup> reveals that the maximum impact of losartan therapy on ACE activity is observed in macroalbuminuric patients with the DD genotype, rather than in normoalbuminuric patients. Furthermore, Andersen et al.<sup>12</sup> show that long-term treatment with losartan has similar beneficial renoprotective effects on the progression of diabetic nephropathy in hypertensive Type 1 diabetic patients with the *ACE* II and DD genotypes.

The present study aimed to investigate the associations between the *ACE* gene *I/D* polymorphisms and the susceptibility of patients with Type 2 diabetes mellitus (T2DM) to DKD; and to examine the correlation between this gene polymorphism and the efficacy of valsartan in reducing urine protein.

## Subjects and methods

In the present study, we enrolled 128 patients diagnosed with T2DM from 1 December 2013 to 31 October 2014 at the Department of Endocrinology, People's Affiliated Hospital of Jiangsu University, China. There were 54 patients, including 35 of male and 19 of female gender (mean age:  $62.43 \pm 11.79$  years) with DKD (DKD+); and 74 controls, including 54 of male and 20 of female gender (mean age:  $60.31 \pm 11.28$  years) without DKD (DKD–). A detailed history was taken, and physical as well as laboratory examinations were performed on the subjects. Informed written consent was obtained from each individual before participation. The study was approved by the

Ethics Committee of People's Hospital of Jiangsu University. T2DM was diagnosed according to the World Health Organization (WHO) criteria, while DKD was defined as an albumin to creatinine ratio (ACR) > 30 mg/g.<sup>13,14</sup> The 54 patients with DKD who were enrolled in this study fulfilled the inclusion criteria of being > 18 years of age, and of not having undergone ARB nor ACE inhibitor therapy within 4 weeks.

Patients were excluded if they had a history of malignant hypertension, congestive heart failure, renal artery stenosis, a creatinine clearance rate of < 30 mL/min, earlier established persistent erythrocyturia and/or urinary infection, or tumor; and if those of female gender were pregnant or lactating.

The polymorphisms of the *ACE* gene were assayed by polymerase chain reaction (PCR), and the genotype distribution and allele frequency were analyzed. The patients with DKD were further divided into three subgroups based on their genotypes: DD, ID and II. According to conventional diabetes therapy, they received valsartan (80 mg) once a day for 12 weeks. After 12 weeks of treatment, measurements of ACR, serum creatinine (SCr), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were repeated.

The data were presented as mean  $\pm$  SD. One-way analysis of variance (ANOVA) was used to analyze the between-group and within-group differences. The paired Student's *t*-test was employed to assess the differences between the baseline values and the values after treatment with valsartan. Count data were presented as medians (interquartile range (IQR)) and the Mann-Whitney U test was used for between-group comparisons. The genotypes and the *ACE* allele frequencies in DKD+ patients were compared to the controls using the chi-square test. Odds ratios (OR) were calculated as estimates of relative risk for disease and the 95% CIs were obtained. Statistical significance was assumed at  $p < 0.05$ . The statistical software package SPSS version 17.0 was used for all the statistical analyses.

## Results

As shown in Table 1, patients with DKD+ had significantly higher ACR, SBP, DBP, and low-density lipoprotein C (LDL-C) levels than did patients with DKD–; however, their high-density lipoprotein C (HDL-C) levels were significantly higher in DKD– patients ( $p < 0.05$ ).

Table 2 presents the *ACE* genotypes and the allele distribution in DKD+ and DKD– patients. In DKD+ subjects, the frequency of DD, ID and II genotypes were 37.1%, 48.1% and 14.8%, respectively. Among the DKD– subjects, 20.3% showed the DD genotype, the ID genotype was present in 44.6% of the patients, while the II genotype existed in 35.1% of the patients. The frequencies of allele D and I were 61.1% and 38.9%, in the DKD+ patients; and 42.6% and 47.4% in the DKD– subjects, respectively ( $p <$

**Table 1.** Baseline clinical characteristics of the groups DKD+ and DKD–.

Variables	DKD+ (n = 54)	DKD– (n = 74)	p value
Age (years)	62.43 ± 11.79	60.31 ± 11.28	0.331
Gender (M/F)	35/19	54/20	0.338
DM duration <sup>a</sup> (years)	10.00 (1.00–40.00)	12.00 (8.00–19.00)	0.996
BMI (kg/m <sup>2</sup> )	24.52 ± 3.73	24.07 ± 2.78	0.059
SBP (mmHg)	145.85 ± 15.88 <sup>c</sup>	129.50 ± 13.99	0.000
DBP (mmHg)	92.22 ± 9.97 <sup>c</sup>	79.42 ± 10.45	0.000
SCr (umol/L)	87.91 ± 29.40	70.70 ± 17.31	0.277
TC (mmol/L)	4.63 ± 0.98	4.87 ± 1.08	0.206
TG (mmol/L)	2.05 ± 1.35	2.28 ± 1.88	0.448
LDL-C (mmol/L)	3.13 ± 0.91 <sup>c</sup>	2.60 ± 0.79	0.001
HDL-C (mmol/L)	0.95 ± 0.22 <sup>c</sup>	1.23 ± 0.42	0.000
ACR (mg/l/g) <sup>a,b</sup>	225.50 (36.70–650.10) <sup>c</sup>	10.20 (0.03–20.60)	0.000
FBG (mmol/L)	11.53 ± 3.74	12.12 ± 3.14	0.336
HbA <sub>1c</sub> (%)	10.93 ± 3.54	10.62 ± 1.93	0.519

<sup>a</sup>Median (minimum – maximum).

<sup>b</sup>Skewed distribution, were logarithmically transformed.

<sup>c</sup>p < 0.05 versus DKD–.

ACR: albumin to creatinine ratio; BMI: body mass index; DBP: diastolic blood pressure; DKD: diabetic kidney disease; DM: diabetes mellitus; F: female; FBG: fasting blood glucose; HbA<sub>1c</sub>: glycosylated hemoglobin; HDL-C: high density lipoprotein; LDL-C: low density lipoprotein; M: male; SBP: systolic blood pressure; SCr: serum creatinine; TC: total cholesterol; TG: triglyceride.

**Table 2.** Comparison of genotypes and allele frequency of ACE gene between the DKD+ and DKD– groups (n (%)).

Groups	DD	ACE genotypes		ACE alleles	
		ID	II	D	I
DKD+	20 (37.1%) <sup>a</sup>	26 (48.1%)	8 (14.8%) <sup>a</sup>	63 (61.1%) <sup>a</sup>	85 (38.9%) <sup>a</sup>
DKD–	15 (20.3%)	33 (44.6%)	26 (35.1)	66 (42.6%)	42 (57.4%)

<sup>a</sup>p < 0.05 versus DKD–.

ACE: Angiotensin converting enzyme; DKD: diabetic kidney disease.

0.05). Computation of the OR (95% CI) as an estimate of relative risk for DKD indicated that the individuals with allele D displayed a 2.12-fold increased risk, as compared to allele I (95% CI: 1.279–3.516; *p* < 0.05).

There were no significant differences in the clinical parameters before the treatment with valsartan, among three genotype subgroups in DKD+ (*p* > 0.05; Table 3).

Six cases were eliminated as shedding cases. Following 12 weeks of treatment with valsartan, the mean value of the urinary albumin to creatinine ratio was significantly reduced in the DD genotype and the ID genotype, while the changes in albuminuria in the II genotype remained non-significant (Table 4). Albuminuria decreased from the baseline by 18% (95% CI: 0.276–0.538; *p* < 0.05) in subgroup DD, and by 8.5% (95% CI: 0.125–0.278; *p* < 0.05) in the ID genotype. The valsartan treatment lowered albuminuria in the II subgroup by 0.7% (95% CI: –0.05–0.38; *p* > 0.05). There were significant differences in the reduction of albuminuria among the three different genotypes (*p* < 0.05). The level of SCr remained unchanged in all the examined subgroups (*p* > 0.05).

The mean value of SBP was significantly lowered in the DD and ID genotypes, while the II genotype showed a non-significant reduction. Similar results were obtained for the DBP, which declined from 91.11 ± 9.95 mmHg at baseline, to 78.21 ± 7.96 mmHg during the investigation period in subgroup DD (*p* < 0.05); and from 91.77 ± 8.67 mmHg at baseline to 86.50 ± 9.87 mmHg during the test in subgroup ID (*p* < 0.05). There was a significant difference in the degree of decrease in urinary protein between the two subgroups in the DKD+ group (*p* < 0.05); however, there was no significant reduction in the SBP nor DBP in subgroup II (*p* > 0.05; Table 4).

## Discussion

DKD is a major microvascular complication of diabetes and is commonly associated with ESRD. The RAS may be activated early in the course of diabetes mellitus, and may trigger changes in intraglomerular hemodynamics and structural modifications in the diabetic kidney at both the glomerular and tubulo-interstitial levels.<sup>2,15,16</sup> In patients

**Table 3.** Comparison of baseline clinical characteristics among different genotypes of group DKD+.

Variables	DD (n = 20)	ACE genotypes	II (n = 8)
		ID (n = 26)	
Age (years)	62.31 ± 10.65	62.80 ± 12.94	61.38 ± 9.58
Gender (M/F)	13/7	17/9	5/3
DM duration <sup>a</sup> (years)	11.50 (1.00–30.00)	10.00 (1.00–40.00)	10.00 (5.00–16.00)
BMI (kg/m <sup>2</sup> )	24.70 ± 4.17	24.51 ± 3.41	24.11 ± 3.28
SBP (mmHg)	143.30 ± 13.59	145.00 ± 13.08	155.00 ± 23.45
DBP (mmHg)	91.11 ± 9.95	91.77 ± 8.67	96.50 ± 12.07
TC (mmol/L)	4.61 ± 0.97	4.60 ± 1.04	4.61 ± 0.94
TG (mmol/L)	1.72 ± 0.74	2.31 ± 1.7	1.84 ± 1.19
LDL-C (mmol/L)	2.53 ± 0.89	2.71 ± 0.81	2.36 ± 0.48
HDL-C (mmol/L)	0.92 ± 0.18	0.90 ± 0.25	0.91 ± 0.19
SCr (umol/L)	93.05 ± 35.75	86.00 ± 22.73	81.25 ± 27.01
ACR (mg/g) <sup>a,b</sup>	231.35 (36.70–621.40)	225.50 (42.80–650.10)	248.90 (104.30–401.30)
FPG (mmol/L)	11.90 ± 4.52	10.61 ± 2.91	13.32 ± 3.66
HbA <sub>1c</sub> (%)	10.21 ± 2.49	11.23 ± 4.30	11.56 ± 2.37

<sup>a</sup>Median (minimum – maximum).

<sup>b</sup>Skewed distribution, were logarithmically transformed.

ACR: albumin to creatinine ratio; BMI: body mass index; DBP: diastolic blood pressure; DKD: diabetic kidney disease; DM: diabetes mellitus; F: female; FBG: fasting blood glucose; HbA<sub>1c</sub>: glycosylated hemoglobin; HDL-C: high density lipoprotein; LDL-C: low density lipoprotein; M: male; SBP: systolic blood pressure; SCr: serum creatinine; TC: total cholesterol; TG: triglyceride.

**Table 4.** Comparison of clinical findings among the three genotypes of DKD+ group after 12 weeks of treatment with valsartan.

Variables	DD (n = 17)	ACE genotypes	II (n = 8)
		ID (n = 23)	
TC (mmol/L)	3.96 ± 0.81	4.17 ± 1.00	3.90 ± 0.84
TG (mmol/L)	1.56 ± 0.67	1.99 ± 1.28	1.50 ± 0.87
LDL-C (mmol/L)	2.16 ± 0.74	2.28 ± 0.67	2.04 ± 0.53
HDL-C (mmol/L)	1.09 ± 0.21	1.14 ± 0.33	1.23 ± 0.15
SCr (umol/L)	87.01 ± 23.18	83.60 ± 24.17	83.00 ± 12.69
FPG (mmol/L)	7.78 ± 0.88	7.73 ± 1.02	7.38 ± 1.06
HbA <sub>1c</sub> (%)	8.52 ± 1.17	8.30 ± 1.40	8.48 ± 1.00
ACR (mg/g) <sup>a</sup>	100.70 (3.40420.50) <sup>b</sup>	160.10 (12.10670.020) <sup>b</sup>	247.20 (89.30402.50)
SBP (mmHg)	125.51 ± 14.39 <sup>b</sup>	135.40 ± 9.59 <sup>b</sup>	149.50 ± 18.78
DBP (mmHg)	78.21 ± 7.96 <sup>b</sup>	86.50 ± 9.87 <sup>b</sup>	92.75 ± 13.43
Reduction rate			
ACR (%) (95%CI)	18.00% (0.27–0.53) <sup>c,d</sup>	8.50% (0.12–0.27) <sup>d</sup>	0.70% (–0.05–0.38)
SBP (mmHg)	16.50 ± 3.89 <sup>c,d</sup>	10.10 ± 8.46 <sup>d</sup>	5.51 ± 7.46
DBP (mmHg)	11.10 ± 4.64 <sup>c,d</sup>	5.41 ± 5.12 <sup>d</sup>	3.72 ± 5.49

<sup>a</sup>median (minimum – maximum) were logarithmically transformed.

<sup>b</sup>p < 0.05 versus baseline.

<sup>c</sup>p < 0.05 versus group ID.

<sup>d</sup>p < 0.05 versus subgroup II.

ACR: albumin to creatinine ratio; BMI: body mass index; DBP: diastolic blood pressure; DKD: diabetic kidney disease; DM: diabetes mellitus; F: female; FBG: fasting blood glucose; HbA<sub>1c</sub>: glycosylated hemoglobin; HDL-C: high density lipoprotein; LDL-C: low density lipoprotein; M: male; SBP: systolic blood pressure; SCr: serum creatinine; TC: total cholesterol; TG: triglyceride.

with DKD, a positive association was reported between the *ACE* D allele/homozygous DD genotype and ESRD.<sup>17</sup> In a large meta-analysis, a 1.27-fold increased risk of DKD was detected in the presence of the DD genotype versus the II genotype; therefore, it was suggested that the *ACE*

I/D polymorphism contributes to the development of DKD, especially among Asian T2DM patients.<sup>18</sup>

Studies related to the involvement of the *ACE* gene I/D polymorphism in DKD have often presented contradictory results. Kumar et al.<sup>19</sup> report that there is no significant

association between the *ACE* I/D polymorphisms and DKD in T2DM patients from Northern India. Conversely, Al-Harbi et al.<sup>20</sup> find that there is a strong association of the *ACE* polymorphism with T2DM patients, although it was not confirmed as a risk factor for the development of DKD in the Bahraini population. In our study, the frequency of the D allele in diabetic patients with DKD was significantly higher (61.1%) than in the diabetic patients without nephropathy (42.6%;  $p < 0.05$ ). The presence of the D allele was associated with a 2.12-fold increased risk of DKD ( $p < 0.05$ ). Our results were consistent with the findings in the aforementioned Asian population.<sup>18</sup> Therefore, our data indicated that the *ACE* gene I/D polymorphism might be associated with the development of DKD, and the D allele is likely to be linked to susceptibility to DKD.

Ang II receptor blockers improve markers of kidney disease, and slow the disease progression in diabetic, as well as non-diabetic patients, by having blood pressure-lowering effects and partially by their direct blockade of Ang II.<sup>21</sup>

Losartan has been shown to have the most beneficial effect on patients with the DD and ID genotypes.<sup>22</sup> This could be attributed to decreased plasma ACE activity and/or decreased urinary albumin excretion in these patients.<sup>23</sup> Similarly, the losartan therapy had greater influence on the ACE activity in macroalbuminuric patients with the DD genotype than in normo-albuminuric patients<sup>10</sup>; however, no significant association was observed between the *ACE* I/D polymorphism and the reduction of albuminuria in losartan-treated hypertensive Type 1 diabetic patients.<sup>12</sup>

In our study, valsartan therapy significantly reduced proteinuria in patients with the DD and ID genotypes; however, the anti-proteinuric response in DD genotype patients was significantly higher ( $p < 0.05$ ) than in subjects with the ID genotype. This could again be related to the increased activity of plasma ACE and/or increased excretion of urinary albumin.

Ortlepp et al.<sup>24</sup> have demonstrated that individuals with the D allele in a homozygous condition experience the most noticeable decrease in blood pressure, during treatment with candesartan. Nevertheless, the association of the *ACE* polymorphism with antihypertensive effect of AT1R antagonists has not been clearly observed in many studies. For instance, Nordestgaard et al.<sup>25</sup> and Nakayama et al.<sup>26</sup> report that there is no correlation between the *ACE* I/D polymorphism and an individual's response to losartan therapy.

In the current study, the SBP and DBP reduced significantly in the genotypes DD and ID ( $p < 0.05$ ); conversely, treatment with valsartan produced a decreased BP response if the diabetic patients had the ACE I allele homozygous. Although the reasons for these discrepancies are not well understood, various factors (racial, environmental and regional) may influence the final outcome of this study and

should be taken into consideration. Moreover, other confounding factors, such as changes in antihypertensive treatment, blood pressure and glycemic control can contribute to the progression of nephropathy. Interestingly, several researchers<sup>10,12,19, 20, 22, 24–26</sup> have examined the effects of gene polymorphisms on a single drug; however, combined medication is common in clinical practice. Therefore, the relationship between gene polymorphism and drug interactions needs to be elucidated. The pharmacogenomic strategies pertaining to DKD are of great significance for ascertaining the treatment options. The results of the present study require further confirmation by including large sample sizes and multicenter clinical trials.

## Conclusions

In summary, the presence of the D allele significantly increased the risk of DKD by 2.12-fold, and the *ACE* I/D polymorphism was suggested to be strongly associated with the development of DKD. In addition, our study revealed that there was a better response to the treatment with valsartan in DKD patients with the DD genotype. This could further provide a basis for individualized therapy for DKD.

## Declaration of conflicting interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethical approval

The study was approved by the Ethics Committee of People's Hospital of Jiangsu University, China.

## Informed consent

Written informed consent was obtained from the patients for their anonymized information to be published in this article.

## Guarantor

CW

## Contributorship

YW and WP contributed equally to this work. Conceived and designed the experiments: CW; Performed the experiments: YW and XZ and HQ; Analyzed the data and wrote the manuscript: YW; Revised the manuscript: CW and YW; Performed the analysis with constructive discussions: WP, LW and ZX.

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