Paper

The insertion/deletion polymorphism of the angiotensin-converting enzyme gene is associated with progression, but not development, of albuminuria in Iranian patients with type 2 diabetes

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Abstract

Introduction. The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene has been shown to be associated with a number of complications of type 2 diabetes. Results on the development and progression of albuminuria, however, have remained controversial, with ethnic differences being a potential reason. The present study is the first report to examine Iranian patients.
Methods. Patients (322; 162 males) with type 2 diabetes were categorised in this cross-sectional study into the following groups: normoalbuminuria (n=145), microalbuminuria (n=129) and macroalbuminuria (n=48). ACE gene I/D polymorphism genotypes were determined using the polymerase chain reaction method.
Results. The distribution of ACE genotypes was significantly different among the groups ($p<0.001$), with the II genotype decreasing and the DD genotype increasing in frequency with increasing severity of albuminuria. Multivariate regression analysis showed that the ACE genotype did not change the odds of having microalbuminuria versus normoalbuminuria, while the D allele independently increased the odds of having macroalbuminuria versus microalbuminuria approximately threefold ($p<0.01$).
Conclusions. In Iranian patients with type 2 diabetes, the D allele is associated with progression, but not development, of albuminuria.

Introduction

The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene, first identified by Rigat *et al.*,[1] accounts for about half of the phenotypic variance in serum ACE levels. The DD genotype or the D allele is associated with elevated circulating and tissue ACE activity compared to the I allele.[2] It is also known that both systemic and renal renin-angiotensin systems are hyperactive in diabetic nephropathy,[3] and inhibition of the renin-angiotensin-aldosterone system (RAAS) exerts beneficial effects on renal outcomes in type 2 diabetic patients by delaying the progression of diabetic nephropathy.[4,5]

"Despite the huge amount of studies looking for candidate genes, the ACE gene remains the unique, well-characterised locus clearly associated with pathogenesis and progression of chronic kidney disease".[6] It would therefore be clinically important to know the association between the ACE gene I/D polymorphism and the development and progression of proteinuria in type 2 diabetes. Studies conducted in different populations have yielded conflicting results. In this study, we attempted for the first time with Iranian patients with type 2 diabetes, to evaluate the association between the ACE gene I/D polymorphism and the development/progression of albuminuria.

Materials and methods

Study population

A total of 322 consecutive patients (162 males) with type 2 diabetes and who referred to our outpatient diabetes clinic between March 2006 and March 2007 was enrolled into the study. Exclusion criteria were: non-Caucasian ethnicity, plasma creatinine higher than 2 mg/dl, history of proteinuria before the onset of diabetes, known adrenal disease, and signs/symptoms suggestive of inflammatory renal disease, urinary tract infection, or benign prostatic hyperplasia. Patients suspected of having any kidney disease other than diabetic nephropathy were referred to the nephrology clinic for further evaluation. All patients gave their written informed consent and the local ethics committee at Tehran University of Medical Sciences approved the study protocol.

The following variables were determined for each patient: age, sex, smoking habits, use and duration of use of ACE inhibitors and their dosages, use of angiotensin II receptor blockers, use of beta-blockers, use of glucose-lowering agents (oral agents, insulin), body mass index (BMI) according to Quetelet equation by using BMI = weight in kilograms/height in metres squared, diabetes duration (years; measured from the time of diagnosis), history of coronary artery disease and its complications, blood pressure (systolic and diastolic), haemoglobin A1c (HbA1c), total cholesterol, low-density lipoproteins (LDL-C), high-density lipoproteins (HDL-C), triglycerides (TG), plasma creatinine, 24-h urine albumin excretion rate (UAE), serum ACE activity, and ACE genotype. Normo- (n=145), micro- (n=129) and macroalbuminuria (n=48) were defined as UAE < 30 mg/day, 30–300 mg/day, and > 300 mg/day, respectively. The average of at least two measurements during the past 6 months was used for this purpose. The diagnosis of diabetes was based upon the American Diabetes Association criteria.[7] Blood pressure was measured after at least 5 minutes of rest in the sitting position, by using a standard mercury sphygmomanometer. The average of two measurements made at least 5 minutes apart was used for analysis. Hypertension was defined as a mean systolic blood pressure of > 140 mmHg, mean diastolic blood pressure of > 90 mmHg or taking antihypertensive medications. Volk or ocular lens (+90 or +78) indirect ophthalmoscopy was performed using a slit lamp biomicroscopy (Topcon, Tokyo, Japan) and fluorescein angiography (Topcon) after dilation of the pupils with tropicamid or cyclogil.[8]

ACE gene I/D polymorphism and serum ACE activity

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 in detail elsewhere.[9] 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. Serum ACE levels were measured by a colourimetric method (Turbidometry assay, Modified Liberman Method) using *p*-hydroxyhippuryl-L-histidyl-L-leucine as the substrate.[10,11]

Statistical analysis

Data were analysed with the SPSS statistical programme (SPSS Inc., SPSS/PCþ, Chicago, Illinois, USA). Results are presented as mean±SD or median (interquartile range) as appropriate. All tests were two-sided and $p<0.05$ was considered statistically significant. Significance of differences between group means and medians (for variables with skewed distributions) was tested by the analysis of variance (ANOVA) procedure and the Mann-Whitney U-test, respectively. Differences in proportions were assessed by the chi-square test. Multivariate logistic regression was used to determine the independent association between genotype (DD versus ID and ID versus II) and the severity of albuminuria. The statistically significant variables extracted from the univariate analysis (i.e. plasma creatinine, HbA1c, retinopathy) were included in regression and the adjusted odds ratios were calculated. Associations were expressed as adjusted odds ratio (OR) with 95% confidence interval (95% CI).

Results

There were no significant differences among the groups in sex, age, smoking habits, diabetes duration, use and duration of use of ACE inhibitors and their dosages, use of glucose-lowering agents, BMI, systolic and diastolic blood pressure, prevalence of hypertension, total cholesterol, HDL-C, LDL-C, TG, and history of coronary artery disease. Ten (6.9%), 13 (10.1%), and four (8.5%) patients received angiotension II receptor blockers ($p=0.638$); 13 (9.0%), eight (6.2%), and one (2.1%) patients received diuretics ($p=0.244$); and 22 (15.2%), 17 (13.2%), and four (8.5%) patients used beta-blockers ($p=0.481$) in groups with normo-, micro-, and macroalbuminuria, respectively. This close match among the groups made it possible to perform further analysis without worrying about many potential confounders. However, more severe albuminuria was significantly associated with higher levels of HbA1c ($p<0.018$), higher plasma creatinine ($p<0.001$), higher prevalence of retinopathy ($p<0.001$), and higher levels of ACE activity ($p=0.019$). The distribution of ACE genotypes was significantly different among the groups ($p<0.001$), with the II genotype decreasing and the DD genotype increasing in frequency with increasing severity of albuminuria (table 1). The distribution of genotypes was in Hardy-Weinberg equilibrium.

To determine independent correlates of the severity of albuminuria, variables with statistically significant associations with albuminuria in the univariate analysis were included in a multivariate regression model. The dependent variable in the model was albuminuria category (normo-, micro- or macroalbuminuria), and potential correlates were plasma creatinine, HbA1c, presence of retinopathy, and ACE genotype. Because of

Table 1
Characteristics of the groups with normo-, micro-, and macroalbuminuria.

	All	Normo-	Micro-	Macro-	p
n (%)	322 (100)	145 (45.0)	129 (40.1)	48 (14.9)	-
Males (%)	162 (50.3)	63 (43.4)	73 (56.6)	26 (54.2)	0.080
Age (years; SD)	59.4±8.5	59.8±8.5	59.7±8.2	57.4±9.1	0.205
Smoking (%)	63 (19.6)	29 (20.0)	28 (21.7)	6 (12.5)	0.384
Diabetes duration (years; SD)	12.7 (5.8)	12.7 (5.2)	12.8 (6.3)	12.3 (6.4)	0.854
Use of ACE inhibitors (%)	136 (42.2)	61 (42.1)	56 (43.4)	19 (39.6)	0.899
ACE inhibitor dosage (mg/day)					
Captopril: mean (range)	25.4 (12.5-100)	24.1 (12.5-100)	26.8 (12.5-100)	25.0 (12.5-50)	0.859
Enalapril: mean (range)	6.6 (2.5-20)	6.6 (2.5-15)	6.3 (2.5-20)	7.3 (2.5-12.5)	0.603
ACE inhibitor duration (years; SD)	4.4 (1.8)	4.3 (1.7)	4.5 (1.9)	4.8 (1.7)	0.605
Drugs for diabetes (%)					0.806
Oral	87 (27.0)	38 (26.2)	35 (27.1)	14 (29.2)	
Insulin	34 (10.6)	13 (9.0)	17 (13.2)	4 (8.3)	
Both	186 (57.8)	85 (58.6)	73 (56.6)	28 (58.3)	
None	15 (4.7)	9 (6.2)	4 (3.1)	2 (4.2)	
BMI (kg/m^2; SD)	26.3 (4.3)	26.3 (4.5)	26.4 (4.5)	26.2 (3.5)	0.945
Systolic blood pressure (mmHg; SD)	139.5 (22.0)	140.0 (21.9)	137.7 (20.7)	143.0 (25.5)	0.337
Diastolic blood pressure (mmHg; SD)	86.1 (11.1)	87.0 (11.0)	84.9 (11.1)	86.3 (11.0)	0.289
Hypertension (%)	161 (50.0)	66 (45.5)	67 (51.9)	28 (58.3)	0.260
HbA1c (%; SD)	8.5 (1.9)	8.2 (1.6)	8.7 (2.1)	8.8 (1.8)	0.018*
Total-C (mg/dl; SD)	214.9 (73.6)	211.4 (44.8)	220.2 (88.3)	211.0 (97.6)	0.570
HDL-C (mg/dl; SD)	43.3 (9.6)	43.0 (9.2)	43.2 (9.1)	44.3 (12.1)	0.731
LDL-C (mg/dl; SD)	120.5 (31.6)	116.6 (31.7)	123.9 (30.5)	123.0 (33.6)	0.135
Triglycerides (mg/dl) Median (25%-75%)	165 (125-212)	172 (122-216)	165 (135-219)	152 (104-182)	0.056
Crt (mg/dl; SD)	0.94 (0.39)	0.86 (0.34)	0.94 (0.34)	1.18 (0.55)	<0.001**
Retinopathy (%)	158 (49.1)	29 (20.0)	83 (64.3)	46 (95.8)	<0.001**
CAD (%)	43 (13.4)	19 (13.1)	18 (14.0)	6 (12.5)	0.962
Serum ACE (U/L; SD)	66.0 (22.9)	62.2 (21.6)	68.5 (24.1)	71.0 (22.2)	0.019*
ACE genotype (%)					<0.001**
II	73 (22.7)	42 (29.0)	28 (21.7)	3 (6.3)	
ID	159 (49.4)	75 (51.7)	69 (53.5)	15 (31.3)	
DD	90 (28.0)	28 (19.3)	32 (24.8)	30 (62.5)	

Key: *p<0.05; **p<0.01. ACE = angiotensin-converting enzyme; BMI = body mass index; CAD = coronary artery disease; Crt = creatinine; D = deletion; HbA1c = haemoglobin A1c; HDL-C = high-density lipoproteins; I = insertion; LDL-C = low-density lipoproteins; SD = standard deviation; Total-C = total cholesterol.

the presence of ACE genotypes in the model, serum ACE activity was not included. ACE genotype did not independently change the odds of having microalbuminuria versus normoalbuminuria, while HbA1c (p=0.001) and retinopathy (p<0.001) were significant independent correlates of microalbuminuria. However, the D allele independently increased the odds of having macroalbuminuria versus microalbuminuria approximately threefold (p<0.01). Plasma creatinine (p=0.009) and retinopathy (p=0.001) were other predictors of macroalbuminuria in the latter analysis (table 2). Serum ACE activity was significantly higher in the DD group (81.6±19.0 U/L) than in the ID group (63.5±20.5 U/L) than in the II group (52.2±21.5 U/L) (p<0.001). After controlling for the albuminuria category and presence of retinopathy, the ACE genotype remained a significant correlate of ACE activity (p<0.001).

Next, we opted to see whether genotypes are associated with the severity of proteinuria after ACE inhibitor treatment. The analysis was therefore limited to the subset of patients who received ACE inhibitors (data not shown). There was no statistically significant difference between the II, ID and DD groups in any of the variables, except the following: serum ACE activity and HbA1c were highest in the DD group, followed by the ID and II groups (p<0.001). Albumin excretion increased from a median (interquartile range) of 28 (22–135) mg/day in the II group to 34 (18–89) mg/day in the ID group to 56 (24–276) mg/day

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Table 2
Multivariate regression to find independent correlates of albuminuria.

	Micro- versus normoalbuminuria			Macro- versus microalbuminuria		
	p	Odds ratio	95% CI	p	Odds ratio	95% CI
Plasma creatinine	0.243	1.58	0.73-3.43	0.009**	3.28	1.35-8.00
HbA1c	0.001**	1.29	1.10-1.50	0.445	1.08	0.88-1.32
Retinopathy	<0.001**	8.43	4.73-15.03	0.001**	12.99	2.86-58.82
ACE genotype						
DD versus ID	0.780	1.10	0.56-2.19	0.002**	3.57	1.60-7.96
DD versus II	0.276	1.85	0.70-3.51	<0.001**	9.55	2.49-36.62

Key: **p<0.01. ACE = angiotensin-converting enzyme; D = deletion; HbA1c = haemoglobin A1c; I = insertion.

in the DD group. However, the trend did not reach statistical significance (p=0.185).

Discussion

We and others have previously shown associations between the ACE gene I/D polymorphism and complications of diabetes such as hypertension and proliferative retinopathy.[9,12] In this study, this polymorphism was significantly associated with progression, but not development, of albuminuria in our type 2 diabetic patients. In particular, patients with the DD genotype had nearly a 10-times higher chance of having macro- versus microalbuminuria than those with the II genotype. In the present study, we had the advantage that the three groups of patients were closely matched in a long list of potentially confounding variables. The potential effects of the three variables (plasma creatinine, HbA1c, and presence of retinopathy), which showed significant differences among the groups, were controlled for during analysis.

The ACE II genotype seems to reduce the risk for renal disease in type 1 diabetes.[13-15] In particular, the D allele is an independent risk factor for both the onset and the progression of diabetic nephropathy in type 1 diabetes.[16] It should be noted, however, that there are a few reports in type 1 diabetes without statistically significant results.[17,18] In type 2 diabetes, there are even more controversies. The potential effects of the ACE gene polymorphism can be viewed from two perspectives: 1) development of/susceptibility to albuminuria and 2) severity/progression of albuminuria. To address the former, the best strategy for a cross-sectional study would be to compare patients with micro- to patients with normoalbuminuria, whereas for the latter, comparing patients with macro- versus microalbuminuria would be appropriate. The lack of such a structured approach in a number of available reports is a potential reason for inconsistency of results. Other reasons include ethnic differences, different criteria used for classification of albuminuria, and small sample sizes in some studies.

The results of studies which evaluated the development of/susceptibility to proteinuria have been highly varied. There was no significant difference in genotype distribution between patients with and without nephropathy in two studies on 445 and 658 German type 2 diabetic patients.[17,19] The same result was repeated in a study on 141 Tunisian type 2 diabetic patients,[20] as well as in a large study on 3,139 French patients.[21] Nephropathy was defined as a UAE of greater than 30 mg/day in these studies. Our results are in agreement with these studies. Our sample size was sufficiently large to enable us to detect, with a power of 80%, genetic associations with odds ratios above 1.75 when comparing between the groups with normoalbuminuria and microalbuminuria. The odds ratio we obtained when comparing these two groups regarding ACE genotypes (DD versus II) was relatively high (OR=1.85). Nevertheless, it was above our detection threshold and the association was not statistically significant.

A number of studies has claimed a significant association of the ACE gene I/D polymorphism with the development of albuminuria in type 2 diabetes. The II genotype was more common among patients without nephropathy among 111 Japanese patients. However, the small number of patients without nephropathy (n=31) might have affected these results.[22] In a small but longitudinal study on 50 Japanese patients, the D allele was associated with a higher risk of developing microalbuminuria over 9 years.[23] In a study on 336 Taiwanese patients, the DD genotype was more common among the 179 patients with nephropathy. Nephropathy was defined in the latter study as a UAE of greater than 500 mg/day.[24]

We are aware of only three studies that assessed the relation between the ACE gene I/D polymorphism and the severity/progression of albuminuria. Within the group with nephropathy in the Taiwanese study, the DD genotype was more common among those under dialysis, indirectly suggesting that the same genotype may have a negative impact on progression of nephropathy.[24] The German study on 331 patients with various stages of diabetic nephropathy showed that patients with higher renal risks had a higher frequency of the DD genotype.[19] The Tunisian study, however, failed to achieve significant results when comparing patients with macroalbuminuria to those with microalbuminuria.[20] Our results support the Taiwanese study.

The available literature on the possible effects of the I/D polymorphism on the renal response to ACE inhibitor therapy continues to be controversial.[25] Depending on the population of study and the study design, results vary from better response by I allele carriers[26,27] to better response by D allele carriers.[28-30] In our study, among patients under ACE inhibition, those carrying the D allele had, though non-significantly, more severe albuminuria than those without this allele. We cannot draw firm conclusions about the association between genotypes and response to ACE inhibitors for at least two reasons. First, only 136 patients in our study received ACE inhibitors. Therefore, we might have missed a significant association. Secondly, we did not have data regarding the severity of proteinuria before ACE treatment was initiated. Our previous study indirectly suggested that the D allele worsens the renal response to ACE inhibition in Iranian patients.[31] Further studies are required to resolve the existing controversies.

In conclusion, the ACE gene I/D polymorphism is associated with altered risk of progression, but not development, of albuminuria in Iranian patients with type 2 diabetes. The cross-sectional nature of the present study does not allow us to make conclusions about potential causal relationships, for which longitudinal studies are required.

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