

Association of Angiotensin Converting Enzyme Gene I/D Polymorphism With Type 2 Diabetes Mellitus¹

MIN YANG*, CHANG-CHUN QIU[†], QUN XU[†], AND HONG-DING XIANG^{1,2}

*Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China; [†]National Laboratory of Medical Molecular Biology, School of Basic Medicine, Peking Union Medical College, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005, China

Objective To investigate the association of angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism with type 2 diabetes mellitus (T2DM). **Methods** Two hundred and nine patients with T2DM diagnosed based on the criteria for diabetes mellitus in 1999 by WHO and 221 controls were recruited from general population of Dongcheng District in Beijing. All subjects were genotyped for the I/D polymorphism of ACE gene by PCR-fragment length polymorphism (FLP) assay. Blood pressure, levels of plasma glucose, lipids and serum insulin were determined. Body mass index (BMI), waist-hip ratio (WHR) and homeostasis model assessment-insulin resistance index (HOMA-IR) were calculated. **Results** The genotype frequencies for ACE genes DD, ID, and II were 19.1%, 42.1%, and 38.8% in patients, respectively, and 9.6%, 49.4%, and 41.0% in controls, respectively. The ACE DD genotype frequency was significantly higher in patients than in controls ($\chi^2=7.61$, $P=0.022$). Multivariate logistic regression analysis showed that the ACE DD genotype was a risk factor for T2DM, with the OR of 2.35 (95% CI 1.17-4.71) adjusted for age, sex, BMI, WHR, blood pressure, and serum cholesterol levels. **Conclusion** The ACE DD genotype is associated with the increased susceptibility to type 2 diabetes mellitus.

Key words: Angiotensin-converting enzyme gene; Polymorphism; Diabetes mellitus; Risk factor; Genetics

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common multifactorial disease with genetic predisposition that is strongly influenced by environmental and behavioral factors, such as obesity and sedentary lifestyle. Previous studies have indicated that obesity, central obesity, physical inactivity, and family history of the disease are major risk factors for T2DM^[1]. Furthermore, the high angiotensin II level and low bradykinin level in the renin-angiotensin system (RAS) related to insulin-resistance are risk factors for diabetes mellitus^[2]. Therefore, variants in the genes regulating RAS are potential risk factors for T2DM.

Angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin system, catalyzes the conversion of angiotensin I to angiotensin II in the liver and inactivates bradykinin in many tissues. High ACE activity is associated with high angiotensin II level and low bradykinin level.

The ACE gene, located on chromosome 17q23,

comprises 26 exons and 25 introns. Seventy-six polymorphisms of the ACE gene have been identified, 18 of which are in complete linkage disequilibrium^[3], including ACE gene insertion/deletion (I/D) polymorphism. ACE gene I/D polymorphism, characterized by the presence (insertion) or absence (deletion) of a 287-bp *AluI*-repeat sequence inside intron 16, has been shown to regulate ACE activity^[4], but studies on association of ACE gene I/D polymorphism with T2DM in various populations have yielded conflicting results^[5-7]. Therefore, the association of ACE gene I/D polymorphism with T2DM was investigated further in a Han Chinese population in the present study.

MATERIALS AND METHODS

Subjects

A total of 2778 permanent residents of Han Chinese without known diabetes mellitus in

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²Correspondence should be addressed to Hong-Ding XIANG, Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, 100730, China. E-mail: Xinghd@vip.sohu.net

Biographical note of the first author: Min YANG, female, born in 1975, Ph.D. Candidate, majoring in molecular genetics and molecular epidemiology of type 2 diabetes.

Dongcheng District of Beijing with cluster sampling were screened for diabetes from June 2003 to June 2004 by medical members, and 209 patients with T2DM were identified according to the 1999 WHO criteria^[8], who should at least have one diabetic first-degree relative. Two hundred and twenty-one subjects with normal glucose tolerance matched for age and gender were randomly chosen as controls. This study was approved by the Institute Review Board of Peking Union Medical College Hospital. A written informed consent was obtained from all subjects involved before participation in the study.

Laboratory Examinations and Blood Pressure Measurements

After a 12-h fasting, some laboratory tests were conducted. Plasma levels of total cholesterol (TC) and triglycerides (TG) were determined by enzymatic colorimetric methods and lipoprotein fractions such as low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) separated by ultracentrifugation. 75-g oral glucose tolerance test (OGTT) was performed by determining 0-, 0.5-, 1-, and 2-h blood glucose after glucose load and serum level of insulin was determined at the same time. Homeostasis model assessment-insulin resistance index (HOMA-IR) was calculated by the formula: fasting blood insulin (mU/L) \times fasting blood glucose (mmol/L)/22.5^[9]. Blood pressure was measured in a sitting position on the right arm after an overnight fasting and rest for 10-15 min before measurement. Three measurements were made at 1 min interval. The mean values of the last two measurements were used for analysis. Hypertension was defined as mean systolic blood pressure (SBP) equal to or greater than 140 mmHg and/or mean diastolic blood pressure (DBP) equal to or greater than 90 mmHg or currently with antihypertensive medication.

Other Body Measurements

Height was measured in centimeters for each subject in an upright standing position without shoes, and weight was measured in kilograms for the subject standing without shoes and in light clothing. Body mass index (BMI) was calculated as weight in kilograms over height in meters squared. Overweight was defined as BMI ≥ 24 ^[10]. Waist circumference was measured in centimeters at the midpoint between the bottom and the top of the iliac crest. Hip circumference was measured at the largest posterior extension of the buttocks. Waist-hip ratio (WHR) was calculated as waist over hip circumference. Central obesity was defined as WHR ≥ 0.85 ^[11].

Genetic Analysis

Genomic DNA was isolated from peripheral blood leukocytes by proteinase K and the phenol/chloroform extraction procedure. Genotyping for the ACE I/D polymorphism was performed by polymerase chain reaction-fragment length polymorphism (PCR-FLP) analysis. Polymerase chain reaction (PCR) was performed in a 25 μ L reaction volume containing 100 ng of genomic DNA. The PCR primers were as follows^[12]: sense oligo 5'-CTGGAGACCACTCCCATCCTTTCT-3' and anti-sense oligo 5'-GATGTGGCCATCACATTCGT CAGAT-3'. PCR amplification was carried out in a PTC-200 MJ research peltier thermal cycler (Perkin-Elmer Corp, Foster City, USA) and followed by a prior denaturation at 94°C for 3 min. This was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min with a final extension at 72°C for 5 min. The PCR products were separated and sized by electrophoresis on a 2% agarose gel. The insertion allele (I) was detected as a 478-bp band and the deletion allele (D) was visualized as a 191-bp band. The genotype results were identified by two independent researchers without the knowledge of the case/control status of each subject.

Statistical Analysis

Quantitative data were presented as $\bar{x} \pm s$. To compare continuous variables between T2DM group and control group, *t*-test for independent samples was used. Distribution of the genotype and allele frequencies in T2DM and control groups was compared using chi-square test. The Hardy-Weinberg equilibrium was also tested by Chi-square test. Relationships between the ACE genotypes and clinical variables were compared with one-way analysis of variance (ANOVA). Logistic regression analysis was performed to evaluate the influence of the genotype and other variables on the risk of T2DM. In logistic regression analysis, dummy variables for the genotype were coded as follows: II (reference), ID ($r_1=1$), DD ($r_2=1$); dummy variables for TC were coded as follows: TC < 200 mg/dL (reference), ≥ 200 mg/dL and < 240 mg/dL ($r_1=1$), ≥ 240 mg/dL ($r_2=1$); dummy variables for LDL-C were coded as follows: LDL-C < 100 mg/dL (reference), ≥ 100 mg/dL and < 130 mg/dL ($r_1=1$), ≥ 130 mg/dL and < 160 mg/dL ($r_2=1$), ≥ 160 mg/dL ($r_3=1$); other variables were coded as follows: HDL-C < 40 mg/dL = 1 and HDL-C ≥ 40 mg/dL = 0; TG ≥ 200 mg/dL = 1 and TG < 200 mg/dL = 0; hypertension = 1 and normotension = 0; overweight = 1 and normal weight = 0; central obesity = 1 and normal WHR = 0; and the study groups

were coded as follows: T2DM group=1 and control group=0. A *P* value less than 0.05 was considered statistically significant. All statistical procedures were performed with SAS version 6.12.

RESULTS

Characteristics of Study Population

Comparison of clinical characteristics between the T2DM and control groups revealed that BMI, WHR, SBP, DBP, TC, TG, HDL-C, LDL-C, fasting insulin, and HOMA-IR were significantly higher in T2DM subjects than in controls (Table 1).

TABLE 1

Characteristics of Study Participants ($\bar{x} \pm s$)			
Variables	T2DM	Control	<i>P</i> Value
Subjects (<i>n</i>)	209	221	—
Age (yr)	50.6 ± 8.4	49.5 ± 5.9	0.111
Gender (M/F)	103/106	113/108	0.702
BMI (kg/m ²)	27.3 ± 3.6	23.3 ± 2.9	<0.001
WHR (cm/cm)	0.90 ± 0.07	0.86 ± 0.07	<0.001
SBP (mmHg)	131.1 ± 17.6	114.1 ± 14.9	<0.001
DBP (mmHg)	84.8 ± 11.5	74.7 ± 9.5	<0.001
TC (mg/dL)	210.9 ± 36.7	182.4 ± 29.3	<0.001
TG (mg/dL)	229.8 ± 204.9	109.3 ± 70.7	<0.001
HDL-C (mg/dL)	49.2 ± 10.1	58.7 ± 13.7	<0.001
LDL-C (mg/dL)	145.2 ± 87.6	118.2 ± 27.6	<0.001
Fasting Insulin (μu/L)	19.5 ± 18.8	10.3 ± 7.7	<0.001
HOMA-IR	5.61 ± 3.59	2.33 ± 1.82	<0.001

Note. Quantitative data are presented as $\bar{x} \pm s$.

ACE Genotype Frequency in Type 2 Diabetes Mellitus

Frequencies of the D and I alleles were 37.3% and 62.7%, respectively. Frequencies of the genotypes DD, ID, and II were 14.4%, 45.8%, and 39.8%, respectively. The genotypes were found in Hardy-Weinberg equilibrium ($\chi^2=0.34, P>0.05$).

For the ACE gene I/D polymorphism, distribution of the genotype was significantly different between T2DM patients and controls ($\chi^2=7.61, P=0.022$). As shown in Table 2, frequency of the homozygous DD genotype in T2DM patients was two-fold higher than that in the controls (19.1% vs. 9.6%; OR for DD vs. II, 2.02; 95% CI, 1.11-3.67).

TABLE 2

Distribution of ACE Gene I/D Polymorphism in T2DM Patients and Controls (%)

ACE Genotype	T2DM	Control	OR	OR 95%CI
II	81 (38.8)	90 (41.0)	1.00	—
ID	88 (42.1)	109 (49.4)	0.90	0.59-1.35
DD	40 (19.1)	22 (9.6)	2.02	1.11-3.67
			$\chi^2=7.61$	
			$P=0.022$	
ACE Allele				
I	59.8	65.4	1.00	—
D	40.2	34.6	1.27	0.72-2.26
			$\chi^2=0.67$	
			$P=0.410$	

Note. OR for genotype was calculated with DD vs. II, ID vs. II. OR for allele was calculated with D vs. I.

The study population could also be divided into three groups according to genotypes: DD (*n*=40), ID (*n*=88), and II (*n*=81) in patients and DD (*n*=22), ID (*n*=109), and II (*n*=90) in controls. Patients with the three ACE genotypes did not differ from controls with such genotypes in gender, age, BMI, WHR, TC, HDL-C, LDL-C, TG, fasting plasma glucose (FBG), 2-h plasma glucose (PBG), fasting insulin, and HOMA-IR. However, patients with the genotype DD displayed the highest mean levels of fasting insulin, those with the genotype II displayed the lowest, and those with the genotype ID displayed the intermediate, though there were no statistical differences between them (24.8 ± 21.8, 21.1 ± 16.9, and 16.7 ± 9.0, respectively; *F*=0.83, *P*=0.442).

Risk Factors for Type 2 Diabetes Mellitus

Independent variables such as hypertension, overweight, central obesity, TC, TG, LDL-C, HDL-C and ACE genotype (Table 3) were included in multivariate logistic regression analysis.

Univariate analysis showed that there was a statistically significant difference in HDL-C and TC between T2DM patients and controls (*P*<0.001), but these two variables were found not to be independent risk factors for T2DM in multiple logistic regression analysis. The other six variables were found to be independent risk factors: DD genotype (OR, 2.35; 95% CI 1.17-4.71), hypertension (OR, 4.31; 95%CI 2.37-7.82), overweight (OR, 3.66; 95%CI 2.11-6.34), central obesity (OR, 2.02; 95%CI 1.13-3.60), hypertriglyceridemia (TG ≥ 200 mg/dL) (OR, 4.26; 95%CI, 2.15-8.43), and high LDL-C (LDL-C ≥ 160 mg/dL) that was the most powerful independent predictor for T2DM with an OR of 7.48 (3.56-15.71).

TABLE 3
Results of Multivariate Logistic Regression Analysis of Risk Factors for T2DM*

Variables	Parameter Estimate	OR	OR 95%CI	P Value	Standardized Estimate
DD Genotype	0.85	2.35	1.17-4.71	0.016	0.17
Hypertension	1.46	4.31	2.37-7.82	0.000	0.36
Central Obesity	0.70	2.02	1.13-3.60	0.018	0.18
Overweight	1.30	3.66	2.11-6.34	0.000	0.35
Hypertriglyceridemia	1.45	4.26	2.15-8.43	0.000	0.33
High LDL	2.01	7.48	3.56-15.71	0.000	0.43

Note. *209 T2DM patients and 221 controls. Study groups coded as 0=control group and 1=T2DM group. LDL-C \geq 160 mg/dL as high LDL-C and TG \geq 200 mg/dL as hypertriglyceridemia.

DISCUSSION

The angiotensin-converting enzyme (ACE) plays an important role in the renin-angiotensin system. Changes of serum ACE activity seem to influence the renin-angiotensin system, which consists of endocrine and local systems. High ACE activity is associated with high angiotensin II level and low bradykinin level. In addition to endocrine system, local systems exist in many tissues including skeletal-muscle^[13] and adipose tissue^[14]. The local skeletal-muscle renin-angiotensin systems may modify the use of substrate through a kallikrein-kinin system, where low ACE leads to increased glucose uptake during exercise^[15]. Low ACE activity has been reported to increase skeletal-muscle glucose uptake, insulin sensitivity, glycogen storage, glucose transport, GLUT-4 synthase activity, and hexokinase activity^[16]. The local adipose renin-angiotensin systems may alter substrate mobilization from fat stores. Low ACE activity has been reported to increase insulin-stimulated hexone transport in adipocytes^[17] and insulin suppression of non-esterified fatty-acid flux^[18]. Taken together, these findings seem to indicate that low ACE activity is advantageous to glucose metabolism.

The ACE gene I/D polymorphism was identified in 1990 by Rigat and co-workers^[4]. Their study showed that the activity of circulating ACE depended on the insertion/deletion (I/D) polymorphism. Subjects with the genotype DD displayed the highest mean activity of serum ACE, those with the genotype II displayed the lowest, and those with the genotype ID displayed the intermediate. Since then, it has been speculated that these differences in serum ACE activity associated with the ACE genotype might affect therapeutic response of ACE inhibitors, explaining interindividual variability in cardiovascular or renal response to equivalent doses of ACE inhibitor^[19]. Several groups have investigated

the extent of this effect modification on response to ACE inhibitors for different indications such as hypertension^[20], diabetic nephropathy^[21], and coronary artery disease^[22]. There is, however, inconsistency in trial findings and as a result, the extent of effect modification of this polymorphism remains unclear.

Our present findings suggest that homozygosis for D in the I/D polymorphism of the ACE gene is associated with T2DM. Multivariate logistic regression analysis of major risk factors for T2DM showed that the DD genotype of the ACE gene was a reliable predictor of T2DM (OR, 2.35; 95%CI 1.17-4.71). This result is in good agreement with the findings in association of the administration of an ACE inhibitor (namely, the decrease of ACE activity) with lower rates of new diagnosis of T2DM in high-risk individuals^[23-24].

The wide racial differences in distribution of the ACE gene polymorphism are well known. The results of the present study indicate that the frequency of the I/D polymorphism appears different in Han Chinese, compared with Caucasian populations^[5-6]. In our study, controls and patients were all Han Chinese with the D allele frequency of 37.3%, which is quite different from the D allele frequency in Caucasian populations (52-57%)^[5-6]. Distribution of the allele in our study was found in Hardy-Weinberg equilibrium, indicating that the sample selection is appropriate.

In this study, the levels of fasting insulin in subjects with ACE DD genotype were higher than those in subjects with ACE ID and II genotypes in T2DM group, though there were no statistical differences, suggesting that the increased ACE activity in individuals with the DD genotype might lead to impaired glucose metabolism or insulin resistance and eventually to T2DM. However, we could not find sufficient evidence to support this idea in our study. Thus, at this point the mechanisms involved in the association of the DD genotype with

the increased susceptibility to T2DM are not clear. In order to verify the presumption, we should study the function of ACE gene I/D polymorphism in the future.

In conclusion, T2DM is associated with the I/D polymorphism in the ACE gene. The ACE DD genotype is associated with T2DM in a Han Chinese population. DD genotype and other factors such as hypertension, overweight, central obesity, hypertriglyceridemia, and high serum LDL-C level are independent risk factors for T2DM. The current investigation provides new evidence for the role of the ACE gene in the pathogenesis of T2DM, which is of significant clinical importance.

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