Insertion/Deletion Polymorphism of the Angiotensin I–Converting Enzyme Gene in Patients With Hypertension, Non–Insulin-Dependent Diabetes Mellitus, and Coronary Heart Disease in Taiwan

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An insertion/deletion (I/D) polymorphism of the angiotensin I–converting enzyme (ACE) gene has been identified that determines most of the plasma ACE activity genetically. Association of the D allele with insulin sensitivity and of the D/D genotype with coronary heart disease (CHD) has been reported in various ethnic populations. To study the role of this genetic polymorphism in patients with hypertension, non–insulin-dependent diabetes mellitus (NIDDM), and NIDDM with CHD in a Taiwanese population, we used a polymerase chain reaction (PCR)-based genotyping technique with an insertion-specific primer for confirmation of the I allele. One hundred ninety-seven unrelated normal controls, 67 subjects with hypertension, 107 subjects with NIDDM, and 70 subjects with NIDDM and CHD were recruited for this study; all were Han Chinese. Subjects without a history of diabetes were studied by a standard 75-g oral glucose tolerance test. Hypertension was diagnosed according to the Fifth Joint National Committee criteria, and CHD was confirmed by a history of acute myocardial infarction and coronary angiographic intervention. The frequency of the I allele of the ACE gene in the normal population was 64.2%, which was higher than reported in white populations. The prevalence of the I allele of the ACE gene was not significantly increased in subjects with hypertension (73.1%), NIDDM (62.1%), and NIDDM with CHD (65%) compared with healthy controls. The I allele of the ACE gene did not correlate with demographic and metabolic variables. I/D polymorphism of the ACE gene is not a marker for hypertension, NIDDM, or CHD in this Taiwanese population.

ANGIOTENSIN I–CONVERTING ENZYME ([ACE] EC 3.4.15.1) plays a key role in modulating vascular tone and electrolyte balance by hydrolyzing angiotensin I to angiotensin II, which is a potent vasopressor and aldosterone-stimulating peptide.[1] The individual variation of plasma ACE levels is largely affected by polymorphism of the ACE gene.[2,3] Elevated plasma ACE levels have been demonstrated in subjects with non–insulin-dependent diabetes mellitus (NIDDM) and diabetics with retinopathy and nephropathy.[4-6] However, the causal effect of elevated ACE levels is not clear, since acute infusion of angiotensin II increases insulin sensitivity[7-9] and blockade by ACE inhibitor or by angiotensin II receptor antagonist also improves insulin sensitivity.[10-12] Therefore, the role of ACE and angiotensin II in the pathogenesis of insulin resistance and NIDDM remains elusive.

Although the biological function is not known, the insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene is associated with a subset of clinical disorders with insulin resistance, such as essential hypertension,[13] coronary heart disease (CHD),[14] and diabetic complications.[15-18] Interestingly, subjects with the D/D genotype have a higher ACE level,[2] which in turn produces a higher angiotensin II level, and have been shown to be more insulin-sensitive.[19] Due to a typing technical difference, it is likely that a conventional polymerase chain reaction (PCR) may overestimate the frequency of D alleles and genotypes.[20] Therefore, we investigated ACE genotypes by the modified PCR method with an insertion-specific primer in normal controls and subjects with clinical diseases with insulin resistance to study the correlation of this genetic polymorphism with hypertension and NIDDM with and without CHD in a Taiwanese population.

SUBJECTS AND METHODS

Subjects

The study subjects included 197 unrelated normal controls, 107 subjects with NIDDM, 70 NIDDM subjects with CHD, and 67 subjects with hypertension recruited from among Han Chinese living in Taiwan. The normal controls were recruited from individuals who presented for a general health evaluation at the National Taiwan University Hospital. Inclusion criteria for patients with NIDDM were as follows: (1) a blood glucose level that met World Health Organization (WHO) criteria, or use of sulfonylurea agents or insulin for diabetic control; (2) no insulin therapy needed within 1 year of diagnosis; and (3) no history of diabetic ketoacidosis. The glucose tolerance of subjects without known diabetes was assessed by a standard 75-g oral glucose tolerance test after an overnight fast according to WHO criteria. The diagnosis of hypertension was made according to criteria of the Fifth Joint National Committee on detection, evaluation, and treatment of high blood pressure, or to regular treatment with antihypertensive agents. CHD was diagnosed according to a medical history of myocardial infarction and/or documented by coronary angiography. This study was approved by the institutional human study committee of the National Taiwan University Hospital, and informed consent was obtained from the participants.

Methods

Peripheral blood leukocyte DNA was extracted. The PCR assay for I/D polymorphism of the ACE gene was performed as previously described.[21] Briefly, 0.1 μg genomic DNA was amplified with a forward primer (5′ CTG GAG ACC ACT CCC ATC CTT TCT 3′), a reverse primer (5′ TCG AGA CCA TCC CGG CTA AAA C 3′), and an insertion-specific primer (5′ GAT GTG GCC ATC ACA TTC GTC AGA T3′). Amplification was performed in a final volume of 10 μL

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Table 1. Demographic Data on the Study Subjects

Parameter	Control	HT	NIDDM	NIDDM + CHD
No. of subjects	197	67	107	70
Age (yr)	56 ± 10	62 ± 13	60 ± 14	58 ± 15
BMI (kg/m²)	24.7 ± 3.5	25.2 ± 3.0	25.1 ± 3.1	24.9 ± 3.6
Age of onset of NIDDM (yr)	—	—	51 ± 11	47 ± 10

Abbreviations: HT, hypertensives; BMI, body mass index.

containing 10 pmol of each primer, 2 mmol/L $MgCl_2$, 50 mmol/L KCl, 10 mmol/L Tris HCl, pH 8.3, 0.001% gelatin, 5% dimethylsulfoxide, 0.2 mmol/L of each dNTP, and 0.25 U Taq polymerase. DNA amplification was achieved by an initial denaturation at 94°C for 5 minutes, followed by 30 cycles with denaturation at 94°C for 1 minute, annealing at 64°C for 1 minute, and extension at 72°C for 1 minute, and then final extension at 72°C for 10 minutes. PCR products were subjected to 2% agarose gel electrophoresis.

Statistical Analysis

The significance of the difference in gene frequency between groups was analyzed by a chi-square test. Differences in variables among three genotypes were analyzed by ANOVA. P less than .05 was considered statistically significant.

RESULTS

Demographic characteristics between normal controls, hypertensive subjects, and patients with NIDDM were not different in this study (Table 1). Genotyping of I/D polymorphism was achieved by the modified PCR technique, since the I allele could be detected by the presence of the upper two bands (479 and 277 bp) originating from the two reverse primers, one in exon 17 and one the insertion-specific sequence in intron 16 (Fig 1). The I-allele frequency of the ACE gene in the normal population was 64.2%. The frequencies of the I and D alleles and the genotypes were consistent with the Hardy-Weinberg equilibrium in the healthy control (Table 2). The prevalence of the I allele of the ACE gene was not significantly increased in subjects with hypertension (73.1%), NIDDM (62.1%), and NIDDM with CHD (65%) compared with healthy controls (Table 2). There was no difference in genotypic frequencies of the ACE gene among normal, hypertensive, NIDDM, and

Table 2. Genotypic and Allelic Frequencies of I/D Polymorphism of the ACE Gene Among Different Groups

Genotype/ Allele	Control (n = 197)	HT (n = 67)	NIDDM (n = 107)	NIDDM + CHD (n = 70)
Genotype				
D/D	27 (13.7%)	4 (6.0%)	17 (15.9%)	11 (15.7%)
D/I	87 (44.2%)	28 (41.8%)	47 (43.9%)	27 (38.6%)
I/I	83 (42.1%)	35 (52.2%)	43 (40.2%)	32 (45.7%)
Allele				
D	141 (35.8%)	36 (26.9%)	81 (37.9%)	49 (35%)
I	253 (64.2%)	98 (73.1%)	133 (62.1%)	91 (65%)

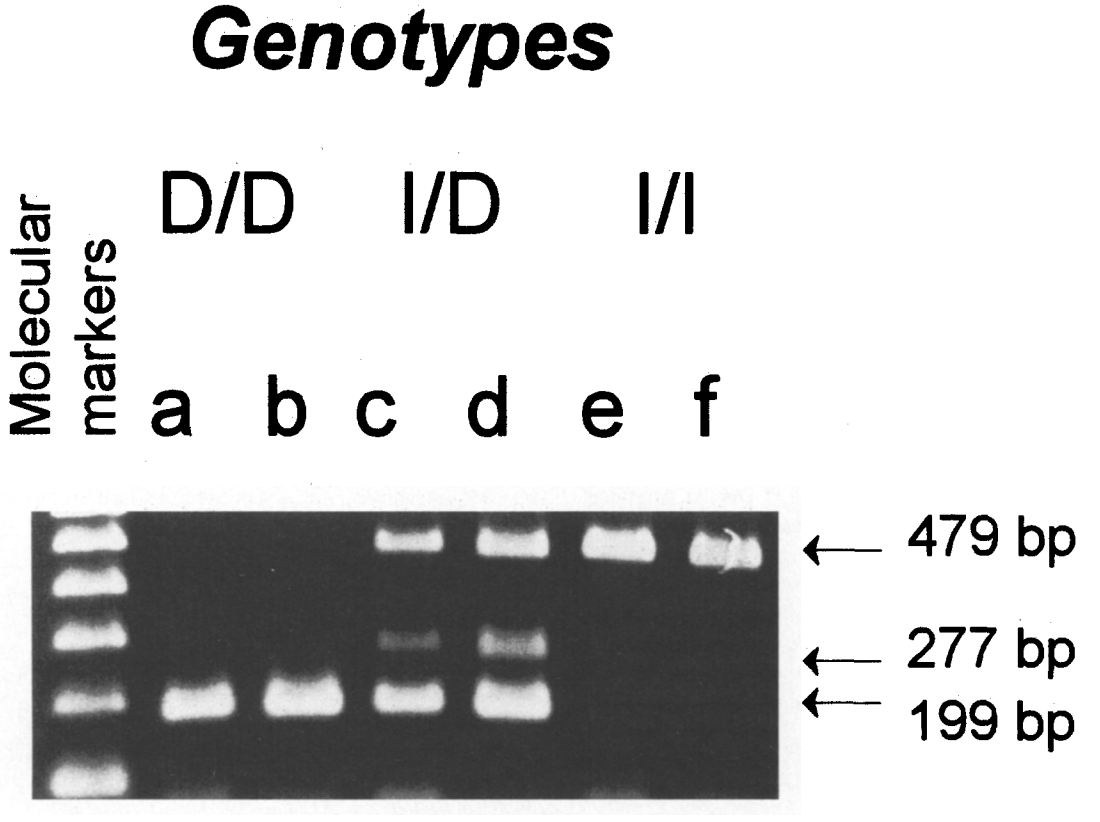

Fig 1. PCR genotyping of I/D polymorphism of the ACE gene. DNA was amplified with 2 standard primers and an insertion-specific primer. DNA fragments were then separated by 2% agarose gel electrophoresis and stained by ethidium bromide. The pGEM 100-bp DNA marker (Promega, Madision, WI) is shown at left. The 479-bp and 277-bp fragments denotes the I allele, and the 199-bp fragment indicates the D allele. Lanes a and b, genotype D/D; c and d, genotype I/D; e and f, genotype I/I.

NIDDM with CHD subgroups (Table 2). To further study the effect of ACE genotype on the clinical manifestation of subjects with NIDDM, we analyzed these variables among three groups of NIDDM subjects with different ACE genotypes by ANOVA (Table 3). There was no significant difference in gender distribution, body build, age at disease onset, or serum cholesterol and triglyceride levels among subjects with different ACE genotypes.

DISCUSSION

The association of I/D polymorphism of the ACE gene with hypertension or NIDDM is conflicting, as reported from different populations.[22-24] We have shown that the frequency of the I allele of the ACE gene was relatively higher in normal Taiwanese compared with caucasians and African-Americans, but similar to values reported in Japanese and lower than in Samoans and Yanomami (Table 4).[17,19,22-26] Interestingly, the incidence and mortality rate of myocardial infarction is higher in Germany than in China,[27] and the CHD incidence is lower in the Paris Prospective Study than in the American population.[28] Moreover, CHD is extremely rare in Samoans and Yanomami.[22] Whether the frequency of CHD is related to D/D genotype frequency in the population deserves further elucidation.

The relationship between insulin resistance and hypertension, NIDDM, and CHD is complex.[29] Our data did not support the notion that the I allele of the ACE gene is a marker for insulin resistance,[19] as we did not observe any differences in body mass index, waist to hip ratio, or serum triglyceride levels among the three groups with different ACE genotypes in our patients with NIDDM. By the association studies in our population, we found that I/D polymorphism of the ACE gene did not play a role in the development of hypertension, NIDDM, and CHD. Recently, we have confirmed that the I/I genotype is associated with a lower plasma ACE activity than I/D and D/D genotypes in the control population.[30] But this association was not observed in the hypertensive subjects, ie, hypertensive subjects with the I/I genotype had a similar level of plasma ACE activity compared with subjects with I/D or D/D genotypes. These data suggest that other mechanisms might operate to maintain higher levels of ACE activity in hypertensive patients with the I/I genotype in contrast to normal controls. Previously, the D allele has been shown to be associated with increased plasma ACE activity but not with an increased risk of CHD in some populations.[26] Taken together, multiple factors other than I/D polymorphism of the ACE gene might be more important for the development of hypertension, NIDDM, and CHD in the Taiwanese population.

In conclusion, I/D polymorphism of the ACE gene does not play an important role in development of hypertension, NIDDM, and CHD in the Taiwanese. This polymorphism is not associated with metabolic factors associated with insulin resistance. Multiple factors other than ACE polymorphism at intron 16 may be involved in the pathogenesis of insulin resistance and associated disorders.

Table 3. Clinical Characteristics of the Subjects According to I/D Polymorphism of the ACE Gene

Variable	ACE Genotype		
	D/D	D/I	I/I
Sex ratio (M:F)	8:9	22:25	20:23
Height (cm)	159 ± 10	159 ± 8	159 ± 9
Weight (kg)	64 ± 10	67 ± 14	65 ± 13
BMI (kg/m^2)	25.4 ± 3.6	25.9 ± 3.8	26.0 ± 4.0
Waist to hip ratio	0.96 ± 0.06	0.94 ± 0.06	0.94 ± 0.07
Age of onset of NIDDM (yr)	56 ± 12	50 ± 12	48 ± 13
Cholesterol (mg/dL)	210 ± 60	200 ± 31	217 ± 48
Triglyceride (mg/dL)	230 ± 180	203 ± 139	203 ± 216

NOTE. Results are the mean ± SD.
Abbreviation: BMI, body mass index.

Table 4. Frequency of Three ACE Genotypes in Normal Populations Among Different Ethnic Groups

Ethnic Group	Frequency of ACE Genotype (%)			Reference No.
	D/D	D/I	I/I	
Taiwanese (n = 197)	13.7	44.2	42.1	Present study
Chinese (n = 189)	9.5	40.7	49.7	25
Japanese (n = 95)	17.9	45.3	36.8	24
French (n = 553)	28.4	53.3	18.3	17
Germans (n = 92)	30.4	55.4	14.1	26
British (n = 533)	32.3	49.2	18.6	19
American whites (n = 139)	30.9	47.5	21.6	23
American blacks (n = 62)	40.3	50	9.7	23
Samoans (n = 58)	1.7	15.5	82.8	22
Yanomami (n = 49)	2.0	26.5	71.4	22

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