Original Article

Angiotensin-Converting Enzyme Gene 2350 G/A Polymorphism Is Associated with Left Ventricular Hypertrophy but Not Essential Hypertension

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The angiotensin-converting enzyme (ACE) gene (*ACE*) is one of the most studied candidate genes related to essential hypertension (EH) and left ventricular hypertrophy (LVH). *ACE* rs4343 synonymous coding polymorphism (2350 G/A) is known among the polymorphisms of this gene to have the most significant effect on plasma ACE concentrations. The aim of the present study was to investigate the association of this polymorphism with EH and LVH in 440 subjects (246 EH patients and 194 controls) from a Chinese Han population. In this study, 2350 G/A genotypes were identified by polymerase chain reaction and restriction digestion in all study participants, and left ventricular mass was assessed by 2-mode echocardiography in 178 untreated EH patients. There was no significant difference in either genotype distribution ($p=0.3659$) or allele frequency ($p=0.1453$) between EH and control groups. In addition, the 2350 G/A polymorphism had no effect on blood pressure in either controls or untreated EH patients. The distribution of genotypes differed significantly when patients with LVH were examined, *i.e.*, 14.71% GG, 54.41% GA, and 30.88% AA patients had this complication, and 36.36% GG, 42.73% GA, and 20.91% AA patients did not ($p=0.0070$). The LVH patients had a higher A allele frequency (58.09%) than patients without LVH (42.27%) ($p=0.0037$). Logistic regression analysis revealed that the association between the A allele and LVH was independent of age, blood pressure, and body mass index. The relative risk of LVH in patients bearing the A allele (GA+AA group) compared with that of GG hypertensive patients was 3.31 (95% confidence interval [CI]: 1.43 to 7.68). These findings suggest an association between LVH and the 2350A allele in hypertensive patients. (*Hypertens Res* 2007; 30: 31–37)

***Key Words*: angiotensin-converting enzyme, genetic polymorphism, essential hypertension, left ventricular hypertrophy**

Introduction

Essential hypertension (EH) is a common disorder in which multiple genetic factors account for 40% of blood pressure variability (*1*). Because the renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure as well as water and sodium balance (*2*), genetic variants in the RAS have been examined as candidates for causing hypertension (*3*). The key enzyme of this system, angiotensin-converting enzyme (ACE), catalyzes the production of angiotensin II, which acts as a strong vasoobliterant and stimulates the secretion of aldosterone (*4*). Published studies have shown that the insertion/deletion (I/D) polymorphism in intron 16 of the

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Table 1. Clinical Characteristics of Hypertensive and Control Subjects

Characteristics	Controls (n=194)	EH (n=246)	p value
Gender (% male)	57.73	55.69	0.6680
Age (years)	53.76±10.04	52.34±9.89	0.1382
BMI (kg/m^2)	23.97±3.04	24.33±3.46	0.2539
SBP (mmHg)	115.39±15.31	159.87±22.53	0.0000
DBP (mmHg)	74.32±8.54	101.61±9.68	0.0000

Values are mean±SD and percentage; EH, essential hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Clinical Characteristics of the Group with LVH as Compared with the Group without LVH in Untreated Hypertensives

Characteristics	LVH(−) (n=110)	LVH(+) (n=68)	p value
Gender (% male)	56.36	55.88	0.9499
Age (years)	52.89±10.17	52.09±9.42	0.6007
BMI (kg/m^2)	24.24±3.31	24.46±3.98	0.6908
SBP (mmHg)	161.94±22.76	163.27±23.14	0.7071
DBP (mmHg)	102.37±10.34	103.15±10.87	0.6322

LVH, left ventricular hypertrophy. Other abbreviations are listed in Table 1.

ACE gene (*ACE*) accounts for approximately half the variance in ACE plasma levels (*5*). Consequently, *ACE* has been postulated as a candidate gene for the development of EH. Zee *et al*. (*6*) first showed that the DD genotype is associated with increased risk of EH in a case-control study in Caucasians, but subsequent studies have yielded conflicting results (*7*).

The physiological function of the I/D polymorphism has not yet been clarified. It is likely that this polymorphism is in strong linkage disequilibrium with another functional mutation within the gene (*8*). Recently, a genome-scan analysis by the Framingham Heart Study group found strong evidence of a quantitative-trait locus (QTL) on chromosome 17, which was close to the *ACE* and was linked to blood pressure (*9*). Among the 13 polymorphisms of the *ACE* that have recently been reported, rs4343 synonymous coding polymorphism (2350 G/A) has been shown to exert the most significant effect on plasma ACE concentrations (*10*).

Based on these findings, we carried out a case-control study of the *ACE* 2350 G/A polymorphism to examine its putative association with EH in a Chinese Han population. We also assessed the association of this polymorphism with hypertensive left ventricular hypertrophy (LVH).

Methods

Study Population

A total of 246 patients with EH (including 178 untreated hypertensive subjects) were eligible for this study. The subjects were enrolled at the First Affiliated Hospital of Soochow University (Suzhou, P.R. China). Hypertension was diagnosed if blood pressure measurements on at least three separate occasions were ≥140 mmHg for systolic and/or ≥90 mmHg for diastolic blood pressure, or when the participant was taking antihypertensive agents. Subjects with secondary hypertension, diabetes mellitus, valvular disease, or apparent ischemic heart disease were excluded. The controls were 194 age-, gender-, and body mass index (BMI)–matched healthy individuals. The controls had no history of hypertension or diabetes mellitus. Normotension was defined as systolic blood pressure (SBP) <140 mmHg, diastolic blood pressure (DBP) <90 mmHg, and lack of current antihypertensive drug treatment. We also excluded from the control group those subjects whose first-degree relatives had hypertension. All study participants were unrelated Han nationality residents of Suzhou (south of P.R. China). No participants included in the sample admitted to any regular alcohol intake. There was also no history of cigarette smoking among these subjects. The study was approved by the Medical Ethics Committee of Soochow University, and written informed consent was obtained from all participants.

Evaluation of LVH

Two-dimensional–controlled M-mode echocardiograms were recorded with each untreated hypertensive subject in the partial left decubitus position after a resting period of at least 10 min. According to the criteria set by the American Society of Echocardiography (*11*), the following parameters relative to the left ventricle were obtained in a blinded fashion, each as an average of at least 3 measurements: 1) left ventricular end-diastolic diameter (LVEDD), 2) left ventricular end-systolic diameter (LVESD), 3) left ventricular diastolic posterior wall thickness (LVDPWT), 4) interventricular septum thickness (IST), and ejection fraction (EF) measured according to the Teicholz method. Left ventricular mass was determined by the Devereux and Reicheck formula (*12*) and the obtained value was divided by the body surface area in order to calculate the left ventricular mass index (LVMI). LVH was diagnosed if the LVMI exceeded 100 g/m^2 in women and 131 g/m^2 in men (*13*). The relative wall thickness (RWT) was measured by using the standardized formula (*14*): 2 × LVDPWT/LVEDD.

Determination of Genotype

Genomic DNA was extracted from peripheral blood leukocytes by the salting-out method (*15*), with minimal modifications. The 2350 G/A genotype in the *ACE* was determined by polymerase chain reaction (PCR) and restriction fragment

Table 3. Distributions of *ACE* 2350 G/A Genotypes and Alleles in Controls, Hypertensive Group and Its Subgroups

Groups	Genotypes frequencies (*n* (%))			*p* value*	Alleles frequencies (%)		*p* value*
	GG	GA	AA		G	A	
Controls (*n*=194)	66 (34.02)	89 (45.88)	39 (20.10)		56.96	43.04	
EH total (*n*=246)	70 (28.46)	116 (47.15)	60 (24.39)	0.3659	52.03	47.97	0.1453
EH treated (*n*=68)	20 (29.41)	32 (47.06)	16 (23.53)	0.7325	52.94	47.06	0.4168
EH untreated (*n*=178)	50 (28.09)	84 (47.19)	44 (24.72)	0.3738	51.69	48.31	0.1491
LVH(+) (*n*=68)	10 (14.71)	37 (54.41)	21 (30.88)	0.0075	41.91	58.09	0.0025
LVH(–) (*n*=110)	40 (36.36)	47 (42.73)	23 (20.91)	0.8657	57.73	42.27	0.8540

*Compared with controls.

length polymorphism (RFLP) as described previously (*10*), with the following modification: a mismatch guanine was introduced at the 3′ end of the primer sequence, resulting in the amplification of a *Bst*U1 restriction site. A 122-bp PCR amplification product was generated from genomic DNA using the following primers: 5′-CTGACGAATGTGATGGC CGC-3′ (sense) and 5′-TTGATGAGTTCCACGTATTTCG-3′ (antisense). PCR reactions were carried out with 200 ng of genomic DNA in a total volume of 50 µl, containing 1.5 mmol/l Mg^{2+}, 5 µl 10× buffer, 0.02 µmol of each of the four dNTPs, 20 pmol of each of the primers, and 1.5 U of DNA polymerase. Thermal processing was initiated at 95°C for 5 min, and then 35 cycles were carried out at 94°C for 30s, 58°C for 30 s, and 72°C for 30 s. This series of cycles was followed by a final extension at 72°C for 10 min. All PCR reactions were carried out in a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer, Foster City, USA). Twenty microliters of the PCR product were digested using 5 U of *Bst*U1 restriction endonuclease (New England Biolabs, Beverly, USA) in 25-µl volumes at 37°C overnight. Digestion with *Bst*U1 yields 100- and 22-bp fragments when A is at position 2350. The digestion products were then separated by electrophoresis on 4% agarose gel, and were stained with ethidium bromide.

Statistical Analysis

All continuous variables are expressed as the mean±SD. Student's *t*-test and analysis of variance (ANOVA) followed by the Newman-Keuls test were used to compare continuous variables from two groups and multiple groups, respectively. Genotypes and allele frequencies were obtained by direct count. Differences in the distribution of alleles and genotypes between the groups, and deviations from the Hardy-Weinberg equilibrium were assessed by χ^2 test. A logistic regression analysis was performed to assess the influence of several factors involved in the association between the *ACE* 2350G/A polymorphism genotype distribution and LVH. All significant tests were two-tailed and were considered statistically significant at $p<0.05$. SAS software (Version 8, SAS Institute, Cary, USA) was used for all statistical analyses.

Results

The clinical characteristics of all participants enrolled in the study are depicted in Table 1. There were no significant differences in gender, age, or BMI between the hypertensive and control groups. In this study, 178 untreated hypertensives were divided into LVH(+) and LVH(–) groups according to the echocardiographic diagnosis. The two groups had no significant differences in gender, age, or BMI, or in SBP or DBP (Table 2).

Table 3 summarizes the distributions of *ACE* 2350 G/A genotypes and allele frequencies for all groups. The genotype distribution among the subjects was in Hardy-Weinberg equilibrium in both the control group ($\chi^2=0.80$, $p=0.37$) and the EH group ($\chi^2=0.75$, $p=0.39$). All subgroups in the EH group also were within Hardy-Weinberg proportions ($p>0.05$). The distribution of the *ACE* 2350 G/A genotypes (GG, GA, and AA) was 34.02%, 45.88%, and 20.10% for the controls, and 28.46%, 47.15%, and 24.39% for all EH subjects, respectively. The derived allele frequencies for the G and A alleles were 56.96% and 43.04% in the control subjects, and 52.03% and 47.97% in the EH subjects respectively. There were no significant differences in either genotype frequency distribution ($p=0.3659$) or allele frequency distribution ($p=0.1453$) between these two groups, which suggested that the 2350 G/A polymorphism of the *ACE* was not significantly associated with EH. On the other hand, the distribution of genotype frequency in the LVH(+) group showed a significant difference when compared to that of controls ($p=0.0075$), namely, the frequency of the A allele in the LVH(+) group was significantly higher than that of subjects in the control group (58.09% *vs*. 43.04%, $p=0.0025$). Moreover, there were significant differences in both genotype distribution ($\chi^2=9.9238$, $p=0.0070$) and allele frequency distribution ($\chi^2=8.4184$, $p=0.0037$) between the LVH(+) group and the LVH(–) group. The relative risk of LVH in GA+AA patients compared with that of GG patients was 3.31 (95% confidence interval [CI]: 1.43 to 7.68). In addition, in a stepwise logistic regression model using LVH as the dependent variable and age, BMI, blood pressure, and *ACE* 2350 G/A polymorphism genotype as independent variables, the presence of the A

Table 4. SBP and DBP Values According to *ACE* 2350 G/A Genotypes in Controls and Untreated Hypertensives

	SBP (mmHg)	DBP (mmHg)
Controls		
GG (n=66)	114.75±15.10	73.86±8.17
GA (n=89)	115.65±15.37	73.34±8.55
AA (n=39)	115.88±15.42	75.06±8.74
	F=0.09, p=0.9133	F=0.56, p=0.5720
EH untreated		
GG (n=50)	162.14±22.19	102.19±10.14
GA (n=84)	161.31±20.46	102.77±10.59
AA (n=44)	164.97±23.19	104.22±10.94
	F=0.42, p=0.6578	F=0.46, p=0.6319

Abbreviations are listed in Table 1.

Table 5. Echocardiographic Parameters According to *ACE* 2350 G/A Genotypes in Untreated Hypertensives

	GG (n=50)	GA+AA (n=128)	p value
LVEDD (mm)	45.34±3.78	49.52±4.14	0.0000
LVESD (mm)	29.83±0.85	30.09±0.91	0.0828
LVDPWT (mm)	9.98±0.86	10.39±1.03	0.0135
IST (mm)	10.80±0.97	11.11±1.12	0.0871
EF (%)	0.63±0.15	0.62±0.14	0.6752
LVMI (g/m^2)	138.75±8.45	144.86±10.26	0.0002
RWT	0.43±0.04	0.44±0.05	0.2078

LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVDPWT, left ventricular diastolic posterior wall thickness; IST, interventricular septum thickness; EF, ejection fraction; LVMI, left ventricular mass index; RWT, relative wall thickness.

allele (GA+AA genotypes) was the only variable independently associated with LVH (p=0.0035).

The effects of the different genotypes on blood pressure are shown in Table 4. There were no significant differences in SBP or DBP among the three genotypes in the control and untreated EH groups. The effects of different genotypes on echocardiographic parameters in untreated hypertensives are shown in Table 5. Because there were no differences in echocardiographic parameters between GA and AA genotypes, the carriers of the A allele (GA+AA) were pooled into one group. EH patients with the GA+AA genotype had greater left ventricular dimensions and thickness than did patients with the GG genotype. Values of LVEDD (49.52±4.14 *vs*. 45.34±3.78 mm, p=0.0000), LVDPWT (10.39±1.03 *vs*. 9.98±0.86 mm, p=0.0135), and LVMI (144.86±10.26 *vs*. 138.75±8.45 g/m^2, p=0.002) were significantly higher in GA+AA patients compared with those of GG patients.

Discussion

During the past decade, there has been growing interest in *ACE* I/D polymorphism as a potential risk factor for EH, in particular after Rigat *et al*. (*16*) reported that more than half of the variance in plasma ACE levels is under the influence of the I/D polymorphism. Zee *et al*. (*6*) first showed that the DD genotype is associated with increased risk of EH in a case-control study of Caucasians. That report was then followed by a number of studies that either supported (*17–20*) or failed to confirm the original hypothesis (*21*, *22*).

The *ACE* I/D polymorphic locus, identified in a non-coding sequence, is more likely to serve as a genetic marker due to its linkage disequilibrium (LD) with a nearby putative disease-causing locus (*8*). Zhu *et al*. (*10*) genotyped 13 polymorphisms in the *ACE* by performing linkage and association analyses, and they found that the 2350 G/A polymorphism in exon 17 had the most significant effect on the plasma ACE concentration, accounting for 19% of the total variance in ACE plasma levels. After adjustment for the effect of the *ACE* 2350 G/A polymorphism, the I/D polymorphism was no longer associated with ACE levels. Their results implied that I/D polymorphism is in LD with 2350 G/A polymorphism and is therefore unlikely to be a functional mutation.

The association between EH as well as hypertensive LVH and the *ACE* 2350 G/A polymorphism has not been studied in the Chinese population. In the present study, we examined the association of the *ACE* 2350 G/A polymorphism in Chinese Han patients suffering from EH.

In this study, no significant difference was observed in either genotype frequency distribution (p=0.3659) or in allele frequency distribution (p=0.1453) between the control and EH groups, suggesting that the 2350 G/A polymorphism of the *ACE* is not significantly associated with EH. We also found no significant differences in SBP or DBP among the three genotypes of controls and untreated EH subjects. This result was not in agreement with the results of the only reported study carried out in an Emirati sample (*23*), which showed that the *ACE* GG 2350 genotype was positively associated (odds ratio [OR]=1.06–3.07, p=0.02) with EH. The discrepancy is likely due to the heterogeneity of subject backgrounds. In addition, the relatively small sample studied in our report (194 controls and 246 EH patients) and in the previous study (136 controls and 118 EH patients) is also likely to be responsible for the discrepancy. It is of interest that the data obtained from the Emirati population also showed no significant differences in SBP or DBP among the three genotypes. Because EH is a complex trait that is influenced by many factors, further studies will still need to be carried out using larger samples and on groups with different environmental exposure status.

LVH is a major independent risk factor for morbidity and mortality due to cardiovascular disease (*24*). Although blood pressure, stroke volume, and decreases in contractile efficiency are important determinants of LVH (*25*), the patho-

Table 6. Comparison of *ACE* 2350 G/A Genotypes and Alleles Frequencies between Chinese and Emirati Populations

	Chinese		Emirati	
	Controls (n=194)	LVH (n=68)	Controls (n=130)*	LVH (n=50)
GG (n (%))	66 (34.02)	10 (14.71)	59 (45.38)	13 (26.00)
GA (n (%))	89 (45.88)	37 (54.41)	43 (33.08)	25 (50.00)
AA (n (%))	39 (20.10)	21 (30.88)	28 (21.54)	12 (24.00)
G (%)	56.96	41.91	61.92	51.00
A (%)	43.04	58.09	38.08	49.00

*Not in agreement with predicted Hardy-Weinberg equilibrium values (χ^2=11.59, p=0.0007). LVH, left ventricular hypertrophy.

genic mechanism of LVH may be multifactorial, involving both hemodynamic and nonhemodynamic factors, such as the sympathetic nervous system and the RAS (*26*). Neuroendocrine factors such as angiotensin II and bradykinin have also been implicated in the modulation of cardiac growth (*27*). Experimental studies suggest that angiotensin II may stimulate cardiac protein synthesis, whereas bradykinin may have an anti-proliferative effect (*28*). ACE is a key enzyme in the production of angiotensin II as well as in the degradation of bradykinin (*4*). The experimental data reported thus far suggests that ACE plays a role in the pathogenesis of LVH. Moreover, several studies have investigated the influence of genetic background on the variability in left ventricular mass in humans, and the heritability of this trait has been estimated to be between 30% and 70% in different populations (*29*). Saeed *et al*. (*30*) first showed that the *ACE* 2350 G/A polymorphism is associated with increased risk of LVH in a nonhypertensive Emirati population. However, there is still little information about other ethnic groups.

In the present study, significant differences of *ACE* 2350 G/A genotypes and allele frequencies were found between patients with LVH and without this complication in untreated hypertensives. We further demonstrated that EH patients with the GA+AA genotype had greater left ventricular dimensions and thickness than did patients with the GG genotype. The A allele was associated with LVH. A logistic regression analysis showed that the association of the A allele with LVH was independent of age, blood pressure, and BMI. Our data support an earlier report of the association between *ACE* 2350 G/A polymorphism and LVH (*30*).

Table 6 shows a comparison of 2350 G/A genotype and allele frequencies between Chinese and Emirati populations (*30*). There was no significant difference among the healthy controls, either in terms of genotype frequency distribution (p=0.0547) or allele frequency distribution (p=0.2080) between the two populations. However, the observed 2350 G/A genotypes in the Emirati controls were not in agreement with the predicted Hardy-Weinberg equilibrium values. The frequency of the A allele in Chinese healthy controls was significantly higher (p<0.05) than that of a Pakistani sample (0.298) (*31*), but lower (p<0.05) than that of a Caucasian sample (0.513–0.542) (*32*) and an Afro-American sample (0.571–0.696 [http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=4343]). The discrepancies between studies in the distribution of *ACE* 2350 G/A polymorphism may be due to ethnic differences.

Most recently, Duan *et al*. (*33*) reported a synonymous mutation in the human dopamine receptor D2 (DRD2); rather than being "silent," it altered the predicted mRNA folding, and led to a decrease in mRNA stability and translation, and dramatically changed dopamine-induced up-regulation of DRD2 expression. The data obtained in that study were suggestive of a possible role played by certain synonymous mutations on functional effects. However, the *ACE* 2350G/A polymorphism may very well not be the actual functional mutation in this context. Instead, it might be located near another functional mutation, as yet undiscovered, in *ACE* or any other gene that could be in LD with it in this region of chromosome 17. In fact, in our study, the frequency of the A allele of *ACE* 2350G/A in patients with LVH was significantly higher than in the controls, while a previous study of a Nigerian sample showed the G allele of the *ACE* 2350G/A polymorphism is most significantly associated with increased plasma ACE levels (*10*). This discrepancy between genotype and phenotype among different populations was also reported by Zhu *et al*. (*10*). In their study, the alleles of different SNPs in the *ACE* responsible for the ACE concentration in a Nigerian sample had the opposite effects in a European population. These discrepant findings suggest that all of these polymorphisms are not functional variants, but instead are in strong LD with a functional variant. Thus, the mechanisms of the association of the *ACE* 2350G/A polymorphism with LVH and ACE concentration in the Chinese population should be examined in future studies.

In conclusion, our data support that *ACE* 2350 G/A polymorphism is associated with LVH but not EH, and the A allele is an independent risk for LVH in Chinese Han EH patients. Given the inherent limitations of case-control studies and the complex nature of genetic susceptibility for chronic degenerative diseases, further studies will still need to be conducted in individual ethnic groups to verify the relevance of this polymorphism to the development of disease.

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