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CLINICAL SCIENCE

Angiotensin-converting enzyme gene 2350 G/A polymorphism and susceptibility to atrial fibrillation in Han Chinese patients with essential hypertension

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OBJECTIVE: The *angiotensin-converting enzyme* gene is one of the most studied candidate genes related to atrial fibrillation. Among the polymorphisms of the *angiotensin-converting enzyme* gene, the 2350 G/A polymorphism (rs4343) is known to have the most significant effects on the plasma angiotensin-converting enzyme concentration. The aim of the present study was to investigate the association of the *angiotensin-converting enzyme* 2350 G/A polymorphism with atrial fibrillation in Han Chinese patients with essential hypertension.

METHODS: A total of 169 hypertensive patients were eligible for this study. Patients with atrial fibrillation (n = 75) were allocated to the atrial fibrillation group, and 94 subjects without atrial fibrillation were allocated to the control group. The PCR-based restriction fragment length polymorphism technique was used to assess the genotype frequencies.

RESULTS: The distributions of the *angiotensin-converting enzyme* 2350 G/A genotypes (GG, GA, and AA, respectively) were 40.43%, 41.49%, and 18.08% in the controls and 18.67%, 46.67%, and 34.66% in the atrial fibrillation subjects ($p = 0.037$). The frequency of the A allele in the atrial fibrillation group was significantly greater than in the control group (58.00% *vs.* 38.83%, $p = 0.0007$). Compared with the wild-type GG genotype, the GA and AA genotypes had an increased risk for atrial fibrillation. Additionally, atrial fibrillation patients with the AA genotype had greater left atrial dimensions than the patients with the GG or GA genotypes ($p<0.01$ and $p<0.05$, respectively).

CONCLUSIONS: The results obtained in this study indicate that the *angiotensin-converting enzyme* 2350 G/A polymorphism is associated with atrial fibrillation and that the A allele shows an increased risk for atrial fibrillation in Han Chinese patients with essential hypertension.

KEYWORDS: *Angiotensin-converting enzyme*; Genetic polymorphism; Atrial fibrillation; Essential hypertension; China.

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INTRODUCTION

Atrial fibrillation (AF) is a rapidly evolving epidemic, representing a multifactorial, dynamic disorder with serious health consequences (1-6). The prevalence of AF is strongly age-dependent, affecting approximately 0.5% of persons aged 40-50 years old and 5-15% of individuals aged 80 years old (3,7). In China, the morbidity related to AF is 0.77% in the adult population (8). AF not only is an independent risk factor for death but also confers a significant risk of morbidity from stroke associated with cardiogenic thromboembolisms (9,10). Despite current achievements in the pharmacological and non-pharmacological treatments of AF, its management remains a difficult task (2).

Essential hypertension (EH) is the most common cardiac condition associated with AF (11). The risk of AF in EH subjects, compared with normotensive subjects, was increased by 1.9 times in the Framingham Heart Study (12) and by 1.4 times in the Manitoba Follow-up Study (13). However, contemporary approaches to prevent AF in EH

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patients have been limited by inefficacy and intolerance (14).

Recently, the rennin-angiotensin system (RAS) has been implicated in the development of AF (15-17), and the inhibition of RAS activity has been shown to reduce AF vulnerability (18). Therefore, over the past decade, the key enzyme of this system, the angiotensin-converting enzyme (ACE), has become one of the most studied candidate genes in AF. Many studies have attempted to shed some light on the possible association between the *ACE* gene insertion/deletion (I/D) polymorphism and the risk of AF. However, previous reports have shown inconsistent and even contradictory results (2).

The physiological role of the I/D polymorphism has not yet been clarified. This polymorphism is likely in strong linkage disequilibrium with another functional mutation within the gene (19). Recently, a genome-scan analysis by the Framingham Heart Study found strong evidence for a quantitative-trait locus on chromosome 17 that was close to the *ACE* gene (20). Among the 13 polymorphisms of the *ACE* gene recently reported, a dimorphism in exon 17, 2350G/A (rs4343), had the most significant effects on the plasma ACE concentration (21).

Based on these findings, we conducted a case-control study of the *ACE* 2350 G/A polymorphism for a putative association with AF in Han Chinese patients with EH.

MATERIALS AND METHODS

A total of 169 patients with EH were eligible for this study. They were all unrelated Han nationality residents and were enrolled at the Affiliated Hospital of Nantong University. Patients with AF (n = 75) were allocated to the AF group, and 94 subjects without AF were allocated to the control group. EH was defined according to the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) criteria (22). AF was defined according to the European Society of Cardiology (ESC) Guidelines for the management of AF (3), based on the replacement of sinus P waves by rapid oscillations or fibrillatory waves that varied in size, shape, and timing, which were associated with an irregular ventricular response when atrioventricular conduction was intact. The presence of AF was determined based on patient history, followed by serial electrocardiography or ambulatory electrocardiographic monitoring. Details regarding the medical history, family history, and clinical symptoms were obtained from all participants using a standardized questionnaire in addition to information on drug intake and cigarette smoking. Blood pressure, height, weight, and waistline were measured by trained physicians or nurses, according to standardized protocols. Patients were excluded if they had acute coronary syndrome; hypertrophic cardiomyopathy; significant valvular disease; left ventricular dysfunction (ejection fraction <50%); or neoplastic, renal, liver, or thyroid diseases. All study participants were unrelated Han nationality residents. The study was approved by the Medical Ethics Committee of Nantong University, and written informed consent was obtained from all participants.

Venous blood samples were obtained after at least a 10-hour overnight fast. The samples were then centrifuged at 2500 rpm for 30 minutes at 4℃ and immediately stored at -80℃ until analysis. The measurement of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and triglycerides (TG) was performed as described previously (7,19).

Genomic DNA was extracted from peripheral blood leukocytes by the salting-out method, with minimal modifications. The determination of the *ACE* 2350 G/A genotypes was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis, as described previously (19,23,24).

All continuous variables are expressed as means and standard deviations (SDs). Student's t-test and analysis of variance (ANOVA), followed by the Newman-Keuls test, were used to compare continuous variables between two groups and among multiple groups, respectively. The genotype and allele frequencies were obtained by direct counts. Differences in the distributions of the alleles and genotypes between the groups and the deviation from Hardy-Weinberg equilibrium were assessed by the $\chi 2$ test. All significant tests were two-tailed and were considered statistically significant at $p<0.05$. SAS software (version 8, SAS Institute, Cary, NC, USA) was used in all statistical analyses. The present sample size of 169 — 75 AF patients and 94 controls — revealed an 87.28% power to detect a significant association ($\alpha<0.05$) for a 7% prevalence of AF in the population (8) using the dominant inheritance model.

RESULTS

The clinical characteristics of all the participants enrolled in the study are depicted in Table 1. No significant differences were observed between the two groups with regard to age, gender, body mass index (BMI), blood pressure, left ventricular ejection fraction (LVEF), serum lipid levels, diabetes, smoking status, or the use of antihypertensive drugs. However, compared with the controls, the AF patients had larger left atrial dimensions.

Table 2 summarizes the distribution of the *ACE* 2350 G/A genotypes and allele frequencies for the two groups. The genotype distribution among the subjects was in Hardy-Weinberg equilibrium in both the control ($\chi 2 = 1.5071$, $p = 0.2196$) and AF ($\chi 2 = 0.1332$, $p = 0.7151$) groups. The distributions of the *ACE* 2350 G/A genotypes (GG, GA, and AA, respectively) were 40.43%, 41.49%, and 18.08% in the controls and 18.67%, 46.67%, and 34.66% in the AF subjects ($p = 0.037$). The frequency of the A allele in the AF group was significantly greater than in the control group (58.00% *vs.* 38.83%, $p = 0.0007$). Compared with the wild-type GG genotype, the GA genotype had a 2.44-fold increased risk of AF (95% confidence interval [CI] = 1.1346-5.2297, $p = 0.0261$), and the AA genotype had a 4.15-fold increased risk of AF (95% CI = 1.7469-9.8650, $p = 0.0016$). After adjustments for gender, age, BMI, blood pressure, LVEF, plasma lipid parameters, smoking status, prevalence of diabetes, and left atrial dimensions, these associations persisted.

The effects of the different genotypes on the clinical parameters are shown in Table 3. There were no significant differences regarding gender, age, BMI, blood pressure, or serum lipid parameters between the genotypes in the AF and control groups. However, the AF patients with the AA genotype had greater left atrial dimensions than the patients with the GG and GA genotypes ($p<0.01$, $p<0.05$).

DISCUSSION

ACE is a dipeptidyl carboxypeptidase I (EC.3.4.15.1) that activates angiotensin I through cleavage of the

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Table 1 - Clinical characteristics of the AF and control subjects.

Characteristics	AF (n = 75)	Controls (n = 94)	*p*-value
Age (years)	71.63 ± 8.62(63-82)	71.25 ± 8.17(60-85)	0.7708
Gender (% male)	62.67	64.89	0.7646
SBP (mm Hg)	145.33 ± 24.86	143.21 ± 23.75	0.5713
DBP (mm Hg)	81.76 ± 10.37	83.31 ± 11.44	0.3632
BMI (kg/m^2)	23.54 ± 3.37	23.86 ± 3.78	0.5671
LVEF (%)	59.86 ± 6.21	61.27 ± 6.59	0.1536
Left atrial dimension (mm)	48.16 ± 7.13	37.42 ± 6.82	0.0000
TC (mmol/L)	4.86 ± 0.59	4.72 ± 0.51	0.1001
LDL-C (mmol/L)	2.50 ± 0.41	2.54 ± 0.46	0.5566
HDL-C (mmol/L)	1.41 ± 0.25	1.47 ± 0.29	0.1576
TG (mmol/L)	1.60 ± 0.52	1.53 ± 0.48	0.3654
Diabetes mellitus (%)	21.33	24.47	0.6308
Smoking (%)	17.33	11.70	0.2974
Diuretics (%)	38.67	28.72	0.1724
Calcium antagonist (%)	48.00	48.94	0.9037
β-blockers (%)	34.67	26.60	0.2561
ACE inhibitors (%)	50.67	54.26	0.6425
ARB (%)	29.33	32.98	0.6118

AF, atrial fibrillation; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; LVEF, left ventricular ejection fraction; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; ACE, *angiotensin-converting enzyme*; ARB, angiotensin receptor blocker.

carboxyterminal dipeptide into the potent vasoconstrictor angiotensin II (ang II), inactivating the vasodilator peptide bradykinin. Recent evidence has indicated that activation of the RAS pathway plays an important role in the development and perpetuation of AF (15-17). ACE expression was elevated in the atrial biopsies of patients with AF, and angiotensin II concentrations were increased in a rapid ventricular pacing-induced congestive heart failure model of AF (2). Angiotensin II and bradykinin have also been implicated in the modulation of cardiac growth (25). Experimental studies have suggested that angiotensin II might stimulate cardiac protein synthesis, whereas bradykinin may have anti-proliferative effects (26). In addition, clinical evidence has suggested that inhibiting the activity of RAS might suppress AF (18). ACE is a key enzyme in the production of angiotensin II and in the degradation of bradykinin (27). Our previous study, conducted in Chinese EH patients, showed that the *ACE* 2350 G/A polymorphism was associated with left ventricular hypertrophy but not EH (23). These experimental and clinical data were suggestive of a possible role for ACE in AF.

Over the past decade, much attention has been devoted to assessing the role of the *ACE* gene I/D polymorphism in AF after Rigat et al. (28) reported that more than half of the plasma ACE levels among individuals were under the influence of the I/D polymorphism. Yamashita et al. (29) first investigated the *ACE* gene I/D polymorphism in 77 Japanese lone AF patients and found that the distribution of *ACE* genotypes was not significantly different between the patients and healthy controls. The subsequent studies, however, yielded inconsistent and even contradictory results (2).

The *ACE* gene I/D polymorphic locus is located in a non-coding region; therefore, it is more likely to be a genetic marker in linkage disequilibrium with the genuine functional variation locus (23). Zhu et al. (21) conducted a linkage and association analysis of 13 polymorphic loci of the *ACE* gene, demonstrating that the 2350 G/A polymorphic locus at exon 17 had the greatest effect on the plasma ACE levels: approximately 19% of the variations in the plasma ACE levels were associated with this polymorphism. The I/D polymorphism was no longer related to the plasma ACE levels after adjustment for the effects of the ACE 2350 G/A polymorphism. The results indicated that the I/D polymorphism was in linkage disequilibrium with 2350 G/A and not the functional variation locus.

We did not test the linkage disequilibrium between the I/D and 2350 G/A polymorphisms in the present study. However, published studies, conducted in Emirati (30), Punjab (31), and Indian (32) populations, have shown that the ACE I/D and ACE 2350 G/A polymorphisms were in strong linkage disequilibrium. The 2350 G/A polymorphic locus, being located in an intron, is unlikely to influence the

Table 2 - *ACE* 2350G/A genotype and allele frequencies in AF and control subjects.

Genotype frequencies (n,%)	AF (n = 75)	Controls (n = 94)	Odds Ratio (95%CI)	*p*-value	Odds ratio* (95%CI)	*p*-value
GG	14 (18.67)	38 (40.43)	-	-	-	-
GA	35 (46.67)	39 (41.49)	2.4359 (1.1346-5.2297)	0.0261	2.3368 (1.1000-4.9644)	0.0409
AA	26 (34.66)	17 (18.08)	4.1513 (1.7469-9.8650)	0.0016	3.1158 (1.3320-7.2885)	0.0117
Allele frequencies (n, %)						
G	63 (42.00)	115 (61.17)	-	-	-	-
A	87 (58.00)	73 (38.83)	2.1755 (1.4050-3.3685)	0.0007	2.0486 (1.3274-3.1615)	0.0015

AF, atrial fibrillation; CI, confidence interval.
*Adjusted for gender, age, BMI, blood pressure, LVEF, plasma lipid parameters, smoking status, prevalence of diabetes, and left atrial dimensions.

Table 3 - Clinical parameters according to different genotypes in the control and AF groups.

Characteristics	AF				Controls			
	GG(n = 14)	GA(n = 35)	AA (n = 26)	*p*-value	GG(n = 38)	GA(n = 39)	AA(n = 17)	*p*-value
Age (years)	71.46±9.12	71.70±9.14	71.63±9.14	0.9966	71.24±9.08	71.29±9.10	71.17±9.05	0.9989
Gender(% male)	64.29	65.71	57.69	0.8067	63.16	64.10	70.59	0.8594
SBP (mmHg)	145.35±24.90	145.57±24.95	145.00±25.07	0.9961	143.09±23.67	143.39±23.83	143.30±23.76	0.9984
DBP (mmHg)	82.18±10.43	81.62±10.37	81.72±10.41	0.9853	83.28±11.35	83.37±11.38	83.25±11.33	0.9991
BMI (kg/m^2)	23.35±3.09	23.59±3.10	23.59±3.12	0.9666	23.82±3.61	23.88±3.62	23.91±3.69	0.9955
LVEF (%)	59.45±5.96	59.93±6.00	59.98±6.03	0.9606	61.31±6.24	61.20±6.21	61.35±6.25	0.9954
Left atrial dimension (mm)	43.48±6.82 *	47.62±6.88 **	51.43±6.96	0.0031	37.12±6.72	37.31±6.73	38.35±6.77	0.8149
TC (mmol/L)	4.75±0.58	4.78±0.60	5.03±0.66	0.2304	4.74±0.54	4.72±0.54	4.67±0.53	0.9055
LDL-C (mmol/L)	2.52±0.40	2.54±0.40	2.43±0.38	0.5459	2.49±0.42	2.58±0.43	2.57±0.43	0.6234
HDL-C (mmol/L)	1.41±0.25	1.39±0.25	1.44±0.26	0.7489	1.48±0.28	1.45±0.27	1.49±0.29	0.8442
TG (mmol/L)	1.63±0.50	1.63±0.49	1.55±0.48	0.7968	1.61±0.47	1.47±0.44	1.49±0.45	0.3734
Diabetes mellitus (%)	18.75	50.00	31.25	0.9432	43.48	39.13	17.39	0.9422

*$p<0.01$, **$p<0.05$ compared with the AA genotype.

expression of *ACE* mRNA directly or to be a functional variant. This fragment has been hypothesized to also be in linkage disequilibrium with an unknown DNA fragment that acts as a silencer. Therefore, the identification of these putative gene loci, which would be in linkage disequilibrium with the 2350 G/A polymorphism, requires further investigation.

The correlation between the ACE 2350 G/A polymorphism and AF has not yet been studied. In the present study, there was a strong association between the *ACE* 2350 G/A polymorphism and the risk of developing AF in EH patients. Compared with the wild-type GG genotype, the GA and AA genotypes showed 2.44-fold and 4.15-fold increased risks of AF, respectively. In the AF group, AA homozygotes also had greater left atrial dimensions than the GG homozygotes and GA heterozygotes. These findings suggest a strong association between the ACE 2350 G/A polymorphism and the risk of developing AF in Han Chinese patients with EH.

The ACE 2350 G/A polymorphism is a synonymous mutation; this type of mutation has traditionally been regarded as ''silent''. Recently, Duan et al. (33) reported a synonymous mutation of the human dopamine receptor D2 (DRD2). In contrast to the previous idea that this type of mutation was ''silent'', this synonymous mutation could change mRNA folding, leading to a decrease in the stability and transcription level of the mRNA and remarkably up-regulating the DRD2 induced by dopamine. That study indicated that some synonymous mutations might also influence some functional effects. However, the ACE 2350G/A polymorphism might not be the actual functional mutation in this context. It might be located near another functional mutation, as yet undiscovered, in *ACE* or any other gene in this region of chromosome 17 that could be in LD. Thus, the mechanisms of the association of the *ACE* 2350G/A polymorphism with AF and the ACE concentrations should be examined in future studies.

Previous case-control studies have shown that the frequency of the A allele in Chinese healthy controls is 0.417-0.451 (19,23,24). The A allele frequency in Chinese subjects was significantly greater ($p<0.05$) than those in a Pakistani population (0.298) (34) and a Malaysian cohort (0.207) (35) but lower ($p<0.05$) than those in a European sample (0.513–0.542) (36) and an Iranian sample (0.591) (37). These data indicate that different ethnic groups might have different ACE gene distributions (38) and that ethnic variation could play a major role in the genetic regulation of serum ACE activity (24).

This study has several potential limitations. First, we could not exclude the presence of previous asymptomatic AF in the control group because these conclusions were based solely on the medical histories obtained from interviews with the participants. Second, the absence of the assessment of serum ACE levels concordant with the *ACE* 2350G/A polymorphism might have limited the outcomes. Finally, although all the study subjects were from the Han Chinese population and the possibility of ethnicity as a confounding factor could thus be excluded, the association of the *ACE* 2350G/A polymorphism and AF in other populations remains unknown and requires further study.

In conclusion, our data indicate that the *ACE* 2350G/A polymorphism is associated with AF and that the A allele shows an increased risk for AF in Han Chinese patients with EH. The *ACE* 2350 G/A polymorphism should be evaluated in EH patients to quantify the risk of AF and, consequently, to improve efforts for preventing or delaying the myocardial remodeling associated with EH. Given the inherent limitations of case-control studies and the complex nature of genetic susceptibility for chronic degenerative diseases, prospective and interventional clinical studies with larger sample sizes will still need to be conducted in individual ethnic groups to verify our observations.

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■ AUTHOR CONTRIBUTIONS

Jiang MH, Su YM, Pan M and Huang ZW conceived the study. Tang JZ, Shen YB, Deng XT and Wu J collected the data. Yuan DS and Jiang MH performed the statistical analysis. Su YM and Wu J analyzed the data. Jiang MH and Huang ZW wrote the manuscript. Jiang MH, Su YM and Deng XT performed the literature search. Pan M and Huang ZW were responsible for the fund collection.

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