Homozygous Deletion Genotype of Angiotensin Converting Enzyme Confers Protection Against Migraine in Man

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Abstract- Studies have shown that migraine may have a major genetic component. Meanwhile, angiotensin converting enzyme (ACE) gene has been implicated as a genetic factor associated with migraine. We designed a case-control study to investigate the association between ACE and migraine in 240 migraine patients and 200 healthy controls, matched by age and sex. There was no significant difference in allelic frequency (I and D) and genotype polymorphism (DD, DI and II) of the ACE gene in migraine patients and controls. Analysis of the difference in ACE polymorphism stratified by gender revealed that male migraine patients with the homozygote DD genotype (ACE-DD) were significantly fewer than that of male controls (OR=0.331, p=0.045). There was no existence of a difference among the frequency and duration of headache in each subgroup of migraine patients stratified by ACE genotype. Our findings indicate that ACE-DD may have a slight protective effect against migraine in male patients.

Key Words: Angiotensin converting enzyme gene, Association study, Genetic polymorphism, Migraine

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INTRODUCTION

Migraine is a common form of chronic cephalgia syndrome, characterized by a pulsating headache, nausea, vomiting, photophobia, and phonophobia. Approximately 15-20% of the general population in Western countries[(1)] and 9.1% in Taiwan[(2)] experience migrainous headaches. Recent studies strongly suggested that migraine might be associated with genetic factors aggregated in families[(3,4)]. Therefore, searches for genetic prediposition factors to migraine is important because it may be helpful in the identification of subjects at risk.

Circulating angiotensin converting enzyme plays a pivotal role in the renin-angiotensin system by conversion of angiotensin I to angiotensin II and degradation of bradykinin[(5)]. Because angiotensin II is a potent vasoconstrictor and bradykinin is a vasodilator, angiotensin converting enzyme is therefore involved in the vasoconstriction and vascular remodeling[(6)]. In addition, angiotensin converting enzyme can modulate inflammation by degradation of bradykinin[(5)]. There are several

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circumstantial reasons implicating that the enzyme is involved in the pathogenesis of migraine. First, migraine may involve vascular instability and neurogenic inflammation in the meningeal blood vessels[7]. Second, endogenous opioids have been recognized to play an important role in pain syndromes[8] and the angiotensin converting enzyme exerts a catabolic role in the degradation of opiate peptides[9]. Third, a chronobiological investigation revealed that the circadian changes in enzymatic activity of serum ACE are less evident in migaine patients[10].

The angiotensin converting enzyme (ACE) gene is located on chromosome 17q23 and consists of 26 exons and 25 introns. A deletion/insertion (D/I) polymorphism of the gene has been identified in intron 16 of a 287-base pair Alu repeat sequence, resulting in three genotypes (DD, ID and II)[11]. ACE gene polymorphism could be correlated with the circulating angiotensin converting enzyme activity, with higher levels in homozygotes of the deletion[12]. Paterna et al. showed that DD genotype of the ACE gene (ACE-DD) may have an increased risk and a higher frequency of migraine attacks[13]. To understand the ethnicity-specific effects on genetic polymorphism, we performed a case-control study to investigate the association between migraine and ACE polymorphism in a Chinese population in Taiwan.

METHODS

Subjects selected for this genetic study were all of Chinese ancestry (ethnic Han origin) and were recruited from the Outpatient Clinic both of Chushang Show-Chwan Hospital in Nantou and Sin-Lau Hospital in Tainan. Migraine patients included in the study had suffered from headache for at least 1 year and were diagnosed according to the criteria of the International Headache Society[14]. The clinical symptoms such asthe presence of aura, frequency (monthly) and duration (hours per month) of migraine attacks were evaluated. The control healthy volunteers were selected by cluster sampling from the community of Chushang Jenn, Nantou, Taiwan. These volunteers were all interviewed for detailed personal health history and family history of migraines. All the migraine patients and healthy controls must have no kinship and be from completely different families. Informed consent was obtained in accordance with a protocol approved by the Human Subjects Research Ethics Committee of both hospitals.

Genomic DNA was extracted from peripheral whole blood using the IsoQuick Nucleic acid extraction kit (ORCA Research Inc., USA) according to the manufacturer's protocol. In the laboratory, the I/D polymorphism of the ACE gene was identified by conventional polymerase chain reaction (PCR). Methods for the designation of primers and procedures for PCR were carried out as described previously[15]. After PCR, the size of the reaction products was determined by electrophoresis on a 2% agarose gel to detect all three different genotypes at this ACE locus (II, ID, DD). To reduce the incidence of mistyping ID as DD genotype, each DD genotype was verified by a second insertion-specific amplification with a primer pair 5'-TGG GAC CAC AGC GCC CGC CAC TAC-3' and 5'-TCG CCA GCC CTC CCA TGC CCA CCA TAA-3'[16]. Each blood specimen was tested in duplicate to assure the reproducibility of the results.

Statistically, the ACE gene polymorphism in migraine patients and healthy controls were compared by chi-square (χ^2) test. Differences in the frequency and duration of migraine attacks among the three genotypes of ACE were compared using one-way ANOVA. The 2×2 contingency table was analyzed with the Pearson chi-square test or Fisher exact test. A p-value smaller than 0.05 was taken as being significant.

RESULTS

This study included 240 migraine patients and 200 healthy controls of ethnic Chinese. The mean age was 29.2 ± 8.5 years (Mean $\pm$SD., ranging from 10 to 53 years) in migraine patients and 29.2 ± 8.4 years (ranging from 7 to 45 years) in healthy controls. The female/male ratio were 2.39 in migraine patients and 2.38 in control subjects. The allelic frequency and genotype of ACE in the control subjects were consistent with the Hardy-Weinberg equilibrium and similar to those in previous genetic studies of the ACE in Chinese populations[15,17-21].

Table 1. *Frequency of the ACE gene polymorphism in patients with migraines and in control subjects*

	Migraine (n =240, %)	Control (n = 200)	χ^2-statistic	p-value
Genotype				
DD	40 (16.7)	34 (17.0)	0.507	0.776
DI	95 (39.6)	85 (42.5)	-	-
II	105 (43.7)	81 (40.5)	-	-
Allele				
D	175 (36.5)	153 (38.3)	0.300	0.584
I	305 (63.5)	247 (61.8)	-	-

ACE: angiotensin converting enzyme gene, D/I: deletion/insertion polymorphism, χ^2: chi-square test.

Table 2. *Frequency of the ACE polymorphism in patients with migraine and in healthy controls stratified by gender*

	Migraine (%)	Control (%)	χ^2	OR	95% CI	p-value
Female (total)	n = 169	n= 141				
Genotype						
DD	35 (20.7)	23 (16.3)	1.368	-		0.505
DI	64 (37.9)	61 (43.3)	-	-	-	-
II	70 (41.4)	57 (40.4)	-	-	-	-
DD	35 (20.7)	23 (16.3)	0.978	1.3405	0.749-2.397	0.323
DI and II	134 (79.3)	118 (83.7)	-	-	-	
Allele						
D	134 (39.6)	107 (37.9)	0.187	1.074	0.777-1.486	0.665
I	204 (60.4)	175 (62.1)	-	-	-	-
Male (total)	n = 71	n = 59				
Genotype						
DD	5 (7.0)	11 (18.6)	4.119	-	-	0.128
DI	31 (43.7)	24 (40.7)	-	-	-	-
II	35 (49.3)	24 (40.7)	-	-	-	-
DD	5 (7.0)	11 (18.6)	4.019	0.331	0.108-1.014	0.045*
DI and II	66 (93.0)	48 (81.4)	-	-	-	-
Allele						
D	41 (28.9)	46 (39.0)	2.958	0.635	0.378-1.067	0.085
I	101 (71.1)	72 (61.0)	-	-	-	-

OR: odds ratio; CI: confidence interval, for the other abbreviations please refer to Table 1. *p < 0.05

Comparison of ACE gene polymorphism between migraine patients and healthy controls revealed no existence of a statistical difference in the allelic frequency (I and D; OR=0.926, 95% CI=0.704 - 1.219, χ^2=0.300,

Table 3. *OR for the frequency of ACE-DD in relation to migraine risk stratified by gender and/or existence of aura*

Subgroups of migraine	No. of patients	OR	95% CI	p-value
Female migraine[a]	169	1.340	0.749-2.397	0.323
Male migraine[b]	71	0.331	0.108-1.014	0.045*
MWA[c]	91	1.465	0.738-2.905	0.273
MOA[c]	149	1.110	0.603-2.042	0.738
Female migraine and MWA[a]	60	1.620	0.737-3.562	0.287†
Female migraine and MOA[a]	109	1.489	0.748-2.967	0.255
Male migraine and MWA[b]	18	1.091	0.262-4.535	1.000†
Male migraine and MOA[b]	53	0.336	0.069-1.630	0.209†

a: compared to female control subjects; *b*: compared to male control subjects; *c*: compared to total control subjects; *p<0.05; †The stastistics was done by the Fisher exact test; ACE-DD: homozygote DD genotypes of ACE; MWA: migraine with aura; MOA: migraine without aura, for the other abbreviations please refer to Tables 1 and 2.

Table 4. *Summation of the ACE polymorphism between migraine patients and control subjects among different ethnic origins*

No. authors	Population	Migraine patients		Control subjects	
		DD genotype	D-allele	DD genotype	D-allele
1. Paterna et al.[13]	Caucasian	48.3 %	62.4 %	37.3 %	49.7 %
2.	Caucasians[21]				50-58 %
3. This study	Chinese	16.7 %	36.5 %	17.0 %	38.3 %
4.	Chinese[21]				34-42 %

p=0.584) and genotypes (ACE-II, ACE-ID and ACE-DD; χ^2=0.507, p=0.776) (Table 1). Further analysis of the difference in ACE polymorphism between the migraine patients and controls stratified by gender (Table 2) revealed that the homozygosity in male migraine patients were 0.331 times fewer than in male controls (95% CI=0.108-1.014, χ^2=4.019, p=0.045). However, neither allelic frequency nor genotype of the ACE gene in the female migraine patients exhibited significant difference from that of the case-control groups.

Moreover, the migraine patients were stratified into two subgroups (Table 3), namely migraine with aura (MWA, n=78, 32.5%) and migraine without aura (MOA, n=162, 67.5%). In comparison with the control subjects, there was no significant differences of the ACE polymorphism in each subgroup. Finally, migraine patients were further divided into four groups: female with MWA (n=60), female with MOA (n=109), male with MWA (n=18), and male with MOA (n=53). The difference of ACE polymorphism between each subgroup and controls again did not reveal any significance (Table 3). We also estimated the frequency (monthly) and duration (hours per month) of migraine attacks stratified by the ACE genotype (three subgroups, ACE-II, ACE-ID, ACE-DD). Although the frequency and duration of migraine in the ACE-DD group was higher than that of the other two groups, the differences did not reach the significance level (p=0.076 in attack frequency and p=0.275 in duration).

DISCUSSION

Paterna et al. first reported that ACE might indicate genetic susceptibility to migraines in Italian[13]. Results of their study revealed that frequency of the ACE-DD genotype was significantly higher in migraine patients without aura than that in the control group. In addition, migraine attacks happen with a significantly higher frequency in migraine patents with the ACE-DD genotype than that of the ACE-DI and ACE-II genotypes. On the contrary, results of our study revealed a significantly inverse association between the frequency of the ACE-DD genotype and the development of migraine. Male individuals with ACE-DD genotype could have a decreased risk of migraine to 0.331 times of that of non-carriers. Therefore, the ACE-DD genotype seems to con-

fer a modestly protective effect against migraine in the male, at least in our Chinese population.

Several factors may explain the discrepancies between our results and that of Paterna's[13]. Differences in study design, selection of the control group, and sample size may contribute to the apparently different results. Meanwhile, our results could be considered to be false positive because the p-value was only marginally significant. In any case, ethnic origin should be carefully considered in the study of the association between genetic factors and the pathogenesis of disease because there are genetic heterogeneities among different genetic backgrounds. Studies have shown that the prevalence of migraine in Asians is much lower than that in Westerners[1,2,22]. Meanwhile, there are also discrepancies in ACE polymorphism among populations. The frequency of the D allele (50 - 58%) of the ACE polymorphism in the Caucasian populations in healthy subjects is higher than that of the I allele. In contrst, the frequency of the D allele of the ACE polymorphism in Chinese (34 - 42%) is lower than that of the I allele[21]. In Paterna's study in Italians[13], patients with migraine without aura showed higher incidence of ACE-DD (48.3%) than control subjects (37.3%). The frequency of the D allele (62.4%) in patients with migraine without aura was also higher than that in control subjects (49.7%). However, our study in Chinese population showed that the frequencies of the ACE-DD genotype (16.7%) and D allele (36.5%) in migraine patients were insignificantly lower than that in control subjects (17.0% and 38.3%, respectively). These results may demonstrate differences in the association study of ACE gene polymorphism among different populations.

There were also instances of different results from different ethnic groups in similar genetic association studies. For example, the studies of Le Couteur et al. in Australians[23] and Kim et al. in Koreans[24] both revealed that the 11-copy allele of the variable number tandem repeat (VNTR) polymorphism within the dopamine transporter gene (DAT) may confer susceptibility to Parkinson's disease (PD). However, other studies revealed an insignificant association between DAT and PD in English and French populations[25,26]. In this regard, our former study in a Chinese population[27] showed that homozygote 10-copy genotype of the VNTR polymorphism within the DAT may even confer a protective factor for male PD patients.

A variety of evidences suggest a link between female sex hormones and migraine[28]. It has been shown that women with migraines outnumber men by at least a 2:1 ratio[1,2,22]. Changes in sustained estrogen levels in pregnancy and menopause appear to affect headaches[29]. Moreover, hormone replacement with estrogen may exacerbate migraine and oral contraceptives can change the character and frequency of migraine[29]. Because ACE-DD is may be genetically protective factor for migraines in the male in our study, we would propose that gender may modify the ACE-associated migraine risk.

In conclusion, the present case-control study shows that the homozygote DD genotype of ACE may provide protection for the development of migraines in the male. It is advisable to reproduce this finding in different populations to reach a more substantial conclusion. Determination of the effect of ACE on the pathogenesis of migraine also requires further study.

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