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ORIGINAL ARTICLE

The Insertion Polymorphism in Angiotensin-Converting Enzyme Gene Associated With the *APOE* ε4 Allele Increases the Risk of Late-Onset Alzheimer Disease

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Abstract

Several studies have shown that a common insertion (I)/deletion (D) polymorphism of angiotensin-converting enzyme (ACE) gene may confer an increased risk of late-onset Alzheimer disease (LOAD). However, the result has not been replicated by all studies. In order to clarify the role of the polymorphism for the occurrence of LOAD in Chinese and the possibility of a synergistic effect with the apolipoprotein E allele 4 on the risk of Alzheimer disease, we examined the *ACE* and *APOE* genotypes in a Chinese sample consisting of 104 sporadic LOAD patients and 128 healthy controls. An obvious difference of allelic and genotypic distributions of *ACE* I/D polymorphism between cases and controls was observed ($\chi^2 = 6.61$, df = 2, $p = 0.037$ by genotype; $\chi^2 = 4.67$, df = 1, $p = 0.031$ by allele). And *ACE* I allele carriers showed an increased risk for LOAD developing ($\chi^2 = 6.59$, df = 1, $p = 0.01$, OR = 2.91, 95% CI 1.25–6.77). After stratifying by *APOE* ε4 status, the increased LOAD risks associated with I allele carriers only in the *APOE* ε4 noncarriers was seen ($\chi^2 = 4.12$, df = 1, $p = 0.042$). Logistic regression analysis of total subjects demonstrated a more than sevenfold increase in the risk of developing LOAD in subjects carrying both the *ACE* I allele and the *APOE* ε4 (OR = 7.39, 95% CI 2.50–21.89, $p < 0.001$). Our data revealed that *ACE* I/D polymorphism is considered to be an additional risk factor, which has strong synergistic interaction with *APOE* ε4 on the risk of LOAD.

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Index Entries: Late-onset Alzheimer's disease; angiotensin-converting enzyme; ACE; apolipoprotein E; polymorphism; Chinese.

Introduction

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by irreversible cognitive and physical deterioration. Multiple genetic and environmental factors regulate the susceptibility AD. Now it is widely accepted that apolipoprotein E (apoE) on chromosome 19 is the only confirmed susceptibility marker, accounting for about 50% of the susceptibility to late-onset AD

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(LOAD) (Blacker, 1997; Saunders, 1993). Therefore, other unknown genetic risk factors acting either alone or in combination with *APOE* ε4 require investigation. A large number of attempts have been made to search for additional genetic risk factors of AD, and many candidate polymorphisms were found to be associated with AD.

Increasing evidence has revealed that angiotensin-converting enzyme (ACE) might be involved in the pathogenesis of LOAD. ACE was a major component of the renin-angiotensin system (RAS), and the brain RAS was implicated in cognitive processes. More directly, ACE was found to significantly inhibit A β aggregation in a dose–response manner (Hu, 2001). This suggested a role for ACE in the development of AD. One insertion (I) or deletion (D) polymorphism at intron 16 of ACE gene was reported to account for about 50% of the interindividual variability of plasma ACE concentration (Rigat, 1990). Therefore, the *ACE* I/D polymorphism may confer susceptibility to AD. Several studies on the association between the I/D polymorphism and AD led to conflicting results (Carbonell, 2003; Cheng, 2002; Isbir, 2001; Kehoe, 1999; Kolsch, 2005; Monastero, 2002; Palumbo, 1999; Perry, 2001). Both I allele and D allele have been reported as possible risk factors for AD in different populations (Kehoe, 1999; Yang, 2000; Isbir, 2002; Palumbo, 1999; Zhang, 2005). The controversial results may be due to ethnical differences between investigated samples. Meta-analyses of the effect of the *ACE* I/D polymorphism on the risk for AD suggested that I-allele or I/D genotype were associated with risk for AD (Lehmann, 2005; Narain, 2000; Elkins, 2004). Yang et al. first reported that *ACE* I allele might be a risk factor for AD independent of *APOE* genotype in Chinese population (Yang, 2000). However, a recent association analysis suggested that the D allele of *ACE* was a probable risk factor for AD in Chinese (Zhang, 2005). To investigate the role of the *ACE* I/D polymorphism for AD in Chinese and the possibility of a synergistic effect with the *APOE* ε4 on the risk of AD, we examined the *ACE* and *APOE* genotypes in LOAD patients and controls from Guangxi, China.

Materials and Methods

Subjects

In total, 104 sporadic LOAD patients (mean age 79.2 ± 6.3; range 64–97 yr; 46% female) and 128 unrelated healthy controls (mean age 68.1 ± 2.8; range 65–77 yr; 30% female) were recruited from Chinese in Guangxi, China. Individuals affected with AD were diagnosed following the DSM-III-R criteria (American Psychiatric Association, 1994) and clinically examined based on the NINCDS-ADRDA criteria to exclude vascular dementia (McKhann, 1984). All LOAD patients were measured through MRI (Zhang, 2003). Healthy controls were selected by the assessment of a full medical history and a physical examination. Cognitive function was assessed using the Mini Mental State Examination (MMSE).

Laboratory Methods

Genomic DNA was extracted from peripheral blood leukocytes using standard method. The genotypes for *APOE* and *ACE* were determined as previously described (Hu, 2002; Rigat, 1992).

Statistical Analysis

The allelic and genotypic distributions of *APOE* and *ACE* polymorphisms were estimated by allele counting and compared in the LOAD and control groups by χ^2 test. Logistic regression analysis was performed to examine the effect of *APOE* and *ACE* polymorphisms on the risk for LOAD using Statistic Package for the Social Science (SPSS). The criterion for significance was set at $p < 0.05$.

Results

As expected, the distribution of *APOE* genotypes was significantly different between LOAD subjects and controls ($\chi^2 = 12.99$, df = 5, $p = 0.023$). The *APOE* ε4 allele frequency in patients with LOAD was significantly higher than that in controls ($\chi^2 = 9.80$, df = 1, $p = 0.0017$).

Allele and genotype frequencies for ACE gene are shown in Table 1. The distribution of *ACE* genotypes was significantly different between LOAD subjects and controls ($\chi^2 = 6.61$, df = 2, $p = 0.037$). Compared with controls (43.8%), there were higher frequencies of genotype I/I (51.0%) in patients, but the association with LOAD did not reach significance in our sample ($\chi^2 = 1.20$, df = 1, $p = 0.27$). In contrast, a significant increase in the frequency of I-allele carriers was observed in the LOAD group compared with the control group ($\chi^2 = 6.59$, df = 1, $p = 0.01$, OR = 2.91, 95% CI 1.25–6.77). Likewise, the allele I frequency in cases (71.6%) was significantly higher than that in controls (62.1%) ($\chi^2 = 4.67$, df = 1, $p = 0.031$).

When the sample was split on the basis of *APOE* ε4 allele status, the genotype and allele distributions of ACE gene in LOAD subjects and controls in the

Table 1
Genotype and Allele Frequencies of Angiotensin-Converting Enzyme Gene Polymorphism Insertion (I)/Deletion (D) in Late-Onset Alzheimer Disease (LOAD) Patients and Controls

		Genotype frequency (%)			Allele frequency (%)	
Group	No.	I/I	I/C	D/D	I	D
LOAD	104	53 (51.0)	43 (41.3)	8 (7.7)	149 (71.6)	59 (28.4)
Controls	128	56 (43.8)	47 (36.7)	25 (19.5)	159 (62.1)	97 (37.9)
		$\chi^2 = 6.61$, df = 2, $p = 0.037$			$\chi^2 = 4.67$, df = 1, $p = 0.031$	

Table 2
Genotype and Allele Distributions of ACE Gene Polymorphism Insertion (I)/Deletion (D) in *APOE* ε4 Carriers and Noncarriers

		Genotype frequency (%)			Allele frequency (%)	
Group	No.	I/I	I/D	D/D	I	D
APOE ε4 Carriers						
LOAD	28	17 (60.7)	10 (35.7)	1 (3.6)	44 (78.6)	12 (21.4)
Controls	14	6 (42.9)	6 (42.9)	2 (14.3)	18 (64.3)	10 (35.7)
		$\chi^2 = 2.17$, df = 2, $p = 0.34$			$\chi^2 = 1.97$, df = 1, $p = 0.16$	
APOE ε4 Noncarriers						
LOAD	76	36 (47.4)	33 (43.4)	7 (9.2)	105 (69.1)	47 (30.9)
Controls	114	50 (43.8)	41 (36.0)	23 (20.2)	141 (61.8)	87 (38.2)
		$\chi^2 = 4.25$, df = 2, $p = 0.12$			$\chi^2 = 2.09$, df = 1, $p = 0.15$	

two subgroups are shown in Table 2. In *the APOE* ε4 carriers, no statistical differences in genotype or allele frequencies between patients and controls were observed ($\chi^2 = 2.17$, df = 2, $p = 0.34$; χ^2=1.97, df = 1, $p = 0.16$ by allele). The difference in I allele carrier frequency between the two groups also did not reach significance ($\chi^2 = 1.62$, df = 2, $p = 0.20$). It is worth noting that the result may be due to the small number of *APOE* ε4 carriers. In contrast, in the group of *APOE* ε4 noncarriers with AD, the allele carriers were more frequent than in controls ($\chi^2 = 4.12$, df = 1, $p = 0.042$), despite the fact that the genotypic and allelic distributions of ACE gene were not obviously different in both groups.

To investigate the simultaneous effect of *ACE* I allele and *APOE* ε4 on the risk for LOAD, logistic regression analysis was performed, taking as reference subjects who possessed neither *ACE* I allele nor *APOE* ε4 polymorphisms (*see* Table 3). In logistic regression, we found an increased risk of LOAD in the subjects with both I allele and *APOE* ε4 (OR = 7.39, 95% CI 2.50–21.89, $p < 0.001$).

Discussion

The results of this case-control association study demonstrated that *ACE* I allele conferred susceptibility for AD developing in Chinese. These results were consistent with the reports in several different ethnic groups including Chinese population (Cheng, 2002; Kehoe, 1999; Yang, 2000; Kolsch, 2005). Interestingly, this study defined I allele as a risk factor for AD dependent of *APOE* ε4 status.

It has been reported that the I/D polymorphism could influence the plasma ACE activity and alter ACE expression (Rigat, 1990; Suehiro, 2004). The I/I genotype carriers show a lower amount of mRNA and lower ACE protein in blood than carriers of the D/D genotype (Suehiro, 2004). Because ACE could degrade amyloid β protein (Aβ) (Hu, 2001), the *ACE* I allele or I/I genotype may increase risk for AD developing by reducing clearance of Aβ indirectly.

Our study also suggested a synergistic effect between *ACE* I allele and *APOE* ε4 on the risk of AD.

Table 3
Binary Logistic Regression Analysis for the Combined Effects of *ACE* I Allele and *ApoE* ε4 on the Risk for Late-Onset Alzheimer Disease (LOAD)

Apoe ε4/ACE-I	Estimated OR	95% CI	*p* Value
ε4+/I+	7.39	2.50–21.89	<0.001
ε4 +/I–	1.64	0.13–20.94	0.70
ε4 –/I+	2.49	1.01–6.14	0.047
ε4–/I–	Reference	Reference	Reference

ε4+, ε4+carriers; ε4–, ε4 noncarriers; I+, carriers of the II or ID genotypes; I–, carriers of the DD genotype.

Both *ACE* I allele and *APOE* ε4 carriers showed a highly increased risk of LOAD (OR = 7.39, 95% CI 2.50–21.89, $p < 0.001$). Meanwhile, it should be noted that there was no association between ε4+/I– status and AD ($p = 0.70$). This conflicted to the fact that *APOE* ε4 was only accepted genetic risk factor for LOAD. The inconsistent result may be partly caused by a chance effect due to the very small number of I–. Another possible explanation is that the synergistic effect of *ACE* I allele and *APOE* ε4 status on AD was so strong that the effect of *APOE* ε4 (ε4+/I–) could not be observed.

To date, many association studies of *ACE* I/D polymorphism and AD have been carried out in European Caucasians, American Caucasians, African Americans, and East Asians. In conflict with evidence that I allele or I/I genotype conferred susceptibility for AD, D allele or DD genotype showed an association with increased risk for AD based on some of those studies (Isbir, 2001; Palumbo, 1999; Zhang, 2005). Linkage disequilibrium with another nearby polymorphism and chance effect may provide a possible explanation for these confused results.

In conclusion, our data reinforced evidence that *ACE* I allele acts as a possible risk factor for AD in Chinese population. Furthermore, the present study revealed a synergistic effect of *ACE* I allele and *APOE* ε4 carrier status on the risk of LOAD. Because the critical sample size in the case-control was not completely matched for age and sex, further studies in larger population-based cohorts as well as biological functional research were required to clarify the substantial role of *ACE* polymorphism in the etiology of AD.

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