Interaction between apolipoprotein-E and angiotensin-converting enzyme genotype in Alzheimer's disease

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Abstract

Both apolipoprotein-E (apo-E) ε4 allele and angiotensin-converting enzyme (ACE) deletion (D) polymorphism have been associated with a high risk for coronary heart disease. Increased frequency of the ε4 allele has also been reported in patients with late-onset of familial and sporadic Alzheimer's disease (AD). The primary aim of this study is to examine the possible relationship between the ACE gene polymorphism and AD. The second aim of this study is to explore the relation of the ACE and apo-E genotypes with AD. Polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and agarose gel electrophoresis techniques were used to determine the apo-E and ACE genotypes.

The frequencies of ACE D and ACE insertion (I) allele among AD patients and controls were 55.7 percent versus 44.2 percent and 51.7 versus 48.2 percent, respectively. Apo-E allele frequencies in the AD group for ε2, ε3 and ε4 were, 1.7 percent, 96.5 percent, and 1.7 percent, respectively. The apo-E allele frequencies of healthy groups for ε2, ε3 and ε4 were 1 percent, 56 percent, and 1.7 percent, respectively.

In conclusion ACE D and apo ε4 allele were found to be more frequent in patients with Alzheimer's disease than in the control group.

Key words: Alzheimer's disease (AD), angiotensin-converting enzyme (ACE), apolipoprotein-E (apo-E), polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP)

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Introduction

Apolipoprotein-E (apo-E) genotype is a major risk factor for late-onset of familial and sporadic Alzheimer's disease (AD). The association between apo-E ε4 and risk of AD was first reported in 1993,[1] and subsequent confirmation has established the apo-E genotype as the single most

Table 1. Distribution of apo-E genotypes and allele frequencies in Alzheimer's patients and control subjects				
	Alzheimer's disease (n = 35)		Control subjects (n = 29)	
Genotype	**n**	**%**	**n**	**%**
E2/2	-		-	-
E2/3	2	5.7	1	3.4
E2/4	1	2.9	-	-
E3/3	26	74.3	27	93.1
E3/4	5	14.3	1	3.4
E4/4	1	2.9	-	-
Alleles				
ε2	3	4.2	1	1.7
ε3	59	84.2	56	96.5
ε4	8	11.4	1	1.7
Apo-E = Apolipoprotein-E; n = number of individuals				

important genetic determinant of susceptibility to sporadic and late-onset familial AD. However, the precise mechanism of pathogenesis is still unknown.[2-6] Apo-E has a ligand for at least three receptors in the central nervous system (CNS), which are the low-density lipoprotein receptor (LDL-R), very low density lipoprotein receptor (VLDL-R), and very low density lipoprotein-like receptor (LRP).

In histopathological examination of patients with AD, apo-E has been found in senile plaques, neurofibrillary tangles, vascular amyloid, and in some nerve cells, with or without neurofibrillary lesions.[4] The importance of apo-E for the nervous system is suggested by its role in the growth and regeneration of both peripheral and CNS tissues during development and after various types of injury. In the CNS, astrocytes synthesize apo-E in response to injury of brain tissue.[7]

Apo-E has three common alleles,[8] designated as ε2, ε3, and ε4, which code respectively for the isoforms of apo-E2, apo-E3 and apo-E4. These alleles have a major impact on total and LDL cholesterol levels in the serum, which are highly correlated with atherosclerotic cardiovascular disease.[9] Several studies were confirmed that ε4 allele has been associated with ischemic heart disease.[9-11] Sparks *et al.*[12] showed that nondemented patients dying with or as a result of critical coronary artery disease had more abundant senile plaques compared with subjects without heart disease. Kosunen and co-workers[13] also reported the association of apo ε4 allele with coronary atherosclerosis in AD patients.

Angiotensin-converting enzyme (ACE) insertion (I) and deletion (D) polymorphism in the ACE gene is associated with a major genetic effect on the inter-individual variability of plasma ACE concentration[14] and deletion polymorphism has been identified as a risk factor for myocardial infarction and cardiomyopathy.[14-16] The ACE DD genotype has also been reported as more common in subjects with stroke history and AD.[17,18] In addition, ACE activity in the cerebrovascular system is increased by aging and AD.[19,20]

Cardiovascular disease is among the leading causes of death, and genetic factors such as Apo-E and ACE alleles are the leading etiological factors. Therefore, we decided to investigate the possible association of two already characterized candidate genes for coding of the apo-E and ACE genes.

Materials and methods

Subjects

Thirty-five patients with AD (26 female, nine male; mean age: 73.91 ± 7.35) and 29 control subjects (nine

Table 2. Distribution of ACE genotypes and allele frequencies in Alzheimer's patients and control subjects				
	Alzheimer's disease (N = 35)		Control subjects (n = 29)	
Genotype	**n**	**%**	**n**	**%**
DD	8	22.9	9	31.0
II	4	11.4	8	27.6
ID	23	65.7	12	41.4
Alleles				
D	39	55.7	30	51.7
I	31	44.2	28	48.2
ACE: Angiotensin-converting enzyme; D = Deletion; I = Insertion; n = Number of individuals				

female, 20 male; mean age: 73.62 ± 13.63) were included in the study. The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria.[21] Healthy persons were defined as subjects without NINCDS-ADRDA dementia criteria and with integrity of their cognitive functions.

Isolation of DNA

Blood specimens were collected in tubes containing EDTA, and DNA was prepared from leucoycte pellets by SDS lysis amonium acetate extraction and ethanol precipitation.[22]

Polymerase chain reaction (PCR) for Apo-E gene polymorphism

Genomic DNAs (0.5-1.0 µg) were amplified by using a forward primer E1, 5'-ATG GAC GAG ACC ATG AAG GAG TTG AAG-3' (codons 64-72), and a reverse primer E2, 5'-TCG CGG GCC CCG GCC TGG TAC A-3' (codons 161-168). A 1 µl DNA template was added in a 50 µl reaction mixture, consisting of 5 µl 10 × reaction buffer (100 mM Tris-HCl, pH 8.8 at 25oC, 500 mM KCl, 0.8 percent Nonidet P40, 25 mM $MgCl_2$), 1 µl (50 pmol) of each primer, 10 µl dNTP mixture (dATP, dGTP, dCTP, dTTP) (1000 µM); 0.3 µl Thermus Aquaticus (taq) DNA polymerase (MBI Fermentas); and 31.7 µl sterile deionized water, which was added to the DNA template. Then, 25 µl mineral oil was placed over the final PCR reaction mixture. Amplification was achieved by 35 cycles of denaturation (1 min at 96°C), annealing (2 min at 67°C), and extension (2 min at 72°C), followed by extension for five minutes at 72°C. The amplified 314 bp PCR product was digested directly with the restriction enzyme Hha I (10 units/µl). The digested DNAs were separated on 4 percent metaphore agarose gel in 1 × Tris borate EDTA buffer, followed by staining by ethidium bromide solution, and the apo-E polymorphism was typed by visualization under ultraviolet (UV) light and photographed with a camera.[23]

E2/2 homozygote contains 111, 91, 78, 18, and 16 bp fragments, indicating the absence of Hha I restriction site at codon 112 (Cys) and 158 (Cys), while the E4/4 homozygote contains 111, 72, 48, 30, 19, 18, and 16 bp fragments, indicating the presence of Hha I restriction sites at codons 112 (Arg) and 158 (Arg). The most common E3/3 homozygote pattern is composed of 111, 91, 48, 30, 18, and 16 bp fragments, reflecting the absence of Hha I restriction site at codon 112 (Cys) and the presence of this site at codon 158 (Arg).

PCR for ACE gene polymorphism

Template DNA (0.5-1.0 µg) was used in a PCR under stringent conditions to avoid the possibility of false-positive readings for ACE genotyping. Reactions were performed with 10 pmoles of each primer: forward primer 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and reverse primer 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3', in a final volume of 50 µl, containing 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris -HCl (pH:8.4), 0.5 mM each dNTP (MBI Fermentas), 1 unit of taq polymerase (MBI Fermentas). Amplification was carried out in a DNA thermal cycler (MJ Research Techne) for 30 cycles

Table 3. Comparison of apo ε4 and ACE D allele's presence				
	Alzheimer's disease		Control subjects	
	n	%	n	%
Apo ε4 allele				
ε4 (-)	28	80	28	96
ε4 (+)	7	20	1	3.4
ACE D allele				
D (-)	4	11.4	8	27.6
D (+)	31	88.6	21	72.4

Apo-E = Apolipoprotein-E; ACE = Angiotensin-converting enzyme; n = number of individuals; ε4(-) = absence of apo ε4 allele; ε4(+) = presence of apo ε4 allele; $\chi 2 = 3.97$; $p < 0.05$; D(-) = absence of ACE D allele; D(+) = presence of ACE D allele; $\chi 2 = 2.71$; $p = 0.09$

with steps for denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. PCR products were separated on 3 percent agarose gel, and DNA was visualized by ethidium bromide staining.[24] The PCR product is a 190 bp fragment in the absence of deletion (D allele) and 490 bp fragment in the presence of the insertion allele (I allele). Thus, each DNA sample revealed one of three possible patterns after electrophoresis: 490 bp band (genotype II), a 190 bp band (genotype DD), or both 490 and 190 bp band (genotype ID).

Statistical analysis

Statistical analyses were performed by using the SPSS software package, revision 5.0. Differences in the distribution of apo-E and ACE genotypes or alleles between AD cases and controls were tested using the chi-square statistic, respectively. Apo-E and ACE allele frequencies were estimated by gene-counting methods. A value of $p < 0.05$ was considered significant.

Results

There was no significant difference between AD cases and control subjects in terms of age (AD, 73.91 ± 7.35 versus 73.62 ± 13.63). The age at the onset of the disease was 69.97 ± 8.2 years (mean ± SD). The distribution of apo-E and ACE genotypes was in Hardy-Weinberg equilibrium in both AD patients and controls.

The apo-E phenotype distribution and allele frequencies are given in Table 1. The phenotypic profile showed enrichment of the E4/4 and E4/3 phenotypes in AD. There was a significantly higher frequency of the apo ε4 allele in the group of AD patients (0.114) than in the control subjects (0.017) ($\chi^2 = 3.97$; $p < 0.05$); results reported in other studies are also shown (Table 3).

The ACE phenotype distribution and allele frequencies are given in Table 2. There was no difference in the ACE allele distribution between two groups. The phenotypic profile shows enrichment of the ID phenotypes in AD. The frequency of deletion alleles (D) was higher in AD group than control subjects. Statistical analysis (Table 3) showed that values for the AD group did not differ significantly from those in the control group ($\chi^2 = 2.71$; $p = 0.09$).

Table 4 shows the interaction between apo ε4 allele and ACE D allele in AD subjects. All of the carriers of ε4 allele had the ACE D allele. This result indicated a significant interaction between the D allele carrier and the ε4 allele carrier states.

Discussion

Apo-E genotype is a significant risk factor for the development of Alzheimer's disease and the apo-E protein is associated within the senile plaques (SP)[4] and

Table 4. Interaction between apo-E ε4 and ACE D alleles in Alzheimer's patients				
Apo ε4 allele	**ε4 (-)**		**ε4 (+)**	
	n	**%**	**n**	**%**
ACE D allele				
D(-)	4	14.3	-	-
D (+)	24	85.7	7	100

Apo-E = Apolipoprotein-E; ACE = Angiotensin-converting enzyme; n = number of individuals; ε4(-) = absence of apo ε4 allele; ε4(+) = presence of apo ε4 allele; D(-) = absence of ACE D allele; D(+) = presence of ACE D allele; $\chi^2 = 1.12$; $p = 0.28$

neurofibrillary tangles (NFT) of the pathological lesions of AD. The association between apo ε4 and AD appears to be robust.[1-6] Marz *et al.*[25] observed a significant positive correlation between both neurofibrillary changes and A4/β-amyloid deposits and the apo ε4 allele dose, and they concluded that apo ε4 causally contributes to AD. In this study, we observed a higher frequency of apo ε3 allele in the control group (AD: 84.2 percent versus controls: 96.5 percent). The genotype and allele distribution of apo ε4 allele significantly differed in these groups ($p < 0.05$). We found that apo-E3/4 was four times more frequent in patients with AD than the control group (AD: 14.3 percent versus controls: 3.4 percent).

Risk of cardiac disease is increased in patients with AD, and the increased frequency of both diseases has been linked to the presence of apolipoprotein E εt4 allele. Since ACE I/D polymorphism appeared to be associated with cardiac disease, it was important to verify the ACE D allele, which might effect the development of coronary atherosclerosis.[13,16,26] Increased frequency of ACE DD genotypes was found in centenarians, in view of its reported association with myocardial infarction.[27] The ACE DD genotype has also been reported to be more common in subjects with a history of stroke and AD.[17,18] Arregui *et al.* reported that there is an increased frequency in ACE activity in patients with AD.[20]

ACE has other biological functions besides its role on the renin-angiotensin and kallikrein-kinin systems. It also has some effect on endocrine phenomenon. Its ability to cleave neuropeptides, such as encephalin, substance P, and LHRH,[28] and its regional distribution in the brain[19] constitute its neuroendocrine functions. Furthermore, increased ACE activity in the cortex with aging suggests that an adaptive response to demand may occur.[19] ACE may also function as an immunomodulator by helping with the MHC class I complex in cytotoxic T lymphocytes,[29] and its level is also associated with the I/D polymorphism in these cells. These data indicate the potential role of ACE, outside the cardiovascular system, which may well influence survival. In this study, the frequency of the ACE deletion allele (D) has also been found higher in the AD group than in control subjects ($p = 0.09$).

Studies of the relationship between ACE or apo-E polymorphism and cardiovascular disease have reported variable results.[27,30] Therefore, in another study, we compared the allele frequencies of the ACE and apo-E DNA polymorphisms in Alzheimer's subjects. We found that all of the carriers of the apo ε4 allele had the ACE D allele. However, the numbers were relatively small, and this might be open to interpretation, but a clear interrelation between these alleles and AD would seem to exist. Further data are necessary to support this observation of an interaction between the apo ε4 and ACE D allele with the severe course of AD.

Finally, it will be important to check for such associations in populations of different ethnic backgrounds and living in different environments.

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