

Original Articles

Study on the Relationship between Polymorphism in Apolipoprotein E Gene and Korean Ischemic Cerebrovascular Disease Patients

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The association between apolipoprotein E (*apo E*) gene polymorphism and ischemic cerebrovascular disease (ICVD) has been controversial. These controversies may be due to inaccurate classification of patients and ethnic differences. We investigated the association between *apo E* genotypes and ICVD patients by case-control study in a Korean population. The association between *apo E* polymorphism and ICVD was examined in 121 patients with ICVD and 132 controls without ICVD. The E3/E4 phenotype was more frequent in control subjects (23.8%) than in patients (13.0%) ($p < 0.05$). The E2/E3 phenotype was more frequent in patients (14.8%) than in control subjects (10.8%), but the difference was not statistically significant ($p > 0.05$). These results suggest that the E4 allele may be a protective factor against early vascular morbidity, and the E2 allele may be a risk factor for cerebrovascular morbidity. (*Korean J of Oriental Med* 2003;24(4):113-119)

Key Words: polymorphism, apolipoprotein E, ischemic cerebrovascular disease

Introduction

Apolipoprotein E (*apo E*) is a 299 amino-acid protein with a central role in cholesterol transport and lipoprotein metabolism. The gene for *apo E* is located on chromosome 19 in linkage with the genes encoding for other apolipoproteins: apo C-I and C-II and the low-density lipoprotein (LDL) receptor gene. It is polymorphic, with three common alleles, E4, E3, E2,

which code for three major isoforms in plasma designated *apo E4*, *apo E3*, and *apo E2* respectively, resulting in six common genotypes¹⁾. *Apo E3* is the predominant isoform. *Apo E4* differs from E3 by an amino acid substitution at position 112 (cys/arg) and from E2 by a substitution at position 158 (arg/cys). *Apo E3* acts as a ligand for two receptors: the *apo E* or "remnant" receptor, which is specifically hepatic, and the LDL receptor (*apo B/E* receptor). The catabolism of TG-rich lipoproteins appears to be modulated by the affinity of *apo E* for *apo E* or *apo B/E* receptors. *Apo E2* binds defectively to receptors, and this results in an increase in the number of LDL receptors, thereby lowering cholesterol levels. *Apo E4* is not covalently

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bonded to *apo* A-II, and its transfer from high-density lipoproteins (HDL) to TG-rich lipoproteins is enhanced. This accelerates hepatic remnant captivation by *apo* E receptors and downregulates the number of LDL receptors, thereby enhancing cholesterol levels.

Apo E is a key protein modulating the highly atherogenic *apo* B containing lipoproteins²⁾ and is a candidate gene for the development of coronary artery disease (CAD). The E2/E2 genotype was the first to be implicated in premature coronary artery disease²⁾, which resulted in this polymorphism being extensively studied. These studies have not shown any clear relationship with the *apo* E polymorphism and risk of CAD, as in some there was a positive association^{3,4)} yet in others no relationship^{5,6)}. Similarly, the evidence supporting a role for the *apo* E gene polymorphism as a risk factor for stroke is contradictory⁷⁾. These controversies may be due to inaccurate classification of subjects and ethnic differences. Furthermore, genetic risk factors in ischemic cerebrovascular disease (ICVD) have been less studied as compared with those involved in CAD. Therefore, the aim of this study was to compare the prevalence of the three most frequent alleles of *apo* E in a defined group of patients with ischemic cerebrovascular disease with those in a control group.

Materials and Methods

1. Patients

Patients with ICVD (n=121) during acute stage were identified according to well-defined criteria that included computerized tomography scanning (CT), magnetic resonance imaging (MRI), and clinical signs (hemiparesis, hemiplegia, slurred speech, facial palsy, and so forth) at Wonkwang University Hospital in Iksan, Korea. The control group consisted of 132 individuals undergoing routine health screening. None of the controls had a history of ICVD. All cases and

controls (all Korean) gave informed consent before participating in the research protocol, which was approved by the ethics committee of each hospital.

2. Determination of *apo* E genotypes

The blood was stored at -20 °C until it was ready to be extracted. The genomic DNA was extracted by inorganic procedure⁸⁾. The concentration of DNA was estimated by absorbance at 260 nm. The *apo* E polymorphism was detected by PCR amplification⁹⁾.

Briefly, a PCR reaction was carried out in a 20 L volume containing 200 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 200 M of each dNTP, and 1 U of *rTaq* DNA polymerase (Takara, Japan), with 1 M of *apo* E F4/F6 primers (Bioneer, Korea). The primer pairs for each gene were as follows: F4: 5' -ACAGAATTCGCCCGGCTG GTACAC-3' , F6: 5' -TAAGCTTGGCACGGCTGTCCAAGGA-3' .

Amplification conditions were 5 min preincubation step at 95 °C, 40 cycles of denaturation at 94 °C for 40 sec, annealing at 67 °C for 40 sec, and extension at 72 °C for 40 sec. A final extension for 10 min at 72 °C was included (MJ Research). The PCR product was digested for 16 h at 37 °C with 5.5 units *Hha*I in the presence of 2 g bovine serum albumin. PCR products were then separated electrophoretically through 8% polyacrylamide gel with a pGEM DNA marker (Promega, U.S.A.) and the products visualized by ethidium bromide staining (Fig. 3). The following fragments were obtained after restriction enzyme digestion: *apo* E2: 91, 81, 21, 18, 16; *apo* E3: 91, 48, 21, 18, 16; *apo* E4: 72, 48, 33, 21, 19, 18, 16. DNA of a subject with known *apo* E4/E4 genotype was included with each batch as a control to prevent inaccurate typing resulting from an incomplete digest. Genotypes were determined without reference to case or control status.

encoding the E4 isoform after amplification by PCR with F4 and F6 primers (10) and shows the six *HhaI* cleavage sites (GCGC) in the amplified E4 sequence, including *HhaI* sites at codons for arginine residues (GCGC) at positions 112 and 158. The E3 sequence encodes a cysteine residue at position 112 (GTGC), which abolishes the *HhaI* cleavage site in the E4 sequence, resulting in a total of five *HhaI* cleavage sites. The E2 sequence encodes cysteine at positions 112 (GTGC) and 158 (GTGC) that abolish two cleavage sites relative to the E4 sequence, resulting in a total of four *HhaI* cleavage sites (Fig. 2).

Fig. 3 shows gel-separated products of *apo E* amplification and *HhaI* digestion. Namely, with the exception of a shared 38 bp fragment, each genotype possessed unique combinations of *HhaI* fragment sizes. The E2/E2 sample contained 91 and 83 bp *HhaI* fragments reflecting the absence of sites at 112 cys and 158 cys. The E3/E3 sample also contained the 91 bp fragment (112 cys), as well as 48 and 35 bp fragments from cleavage at the *HhaI* site at 158 arg. The E4/E4

sample also contained these 48 and 35 bp fragments (158 arg), as well as a unique 72 bp fragment from cleavage at 112 arg.

2. Clinical characteristics of patients with ICVD

Table 1 shows the clinical characteristics of the present subjects. A total of 121 patients were included in the analysis.

3. Clinical characteristics according to *apo E* genotypes in patients with ICVD

Table 2 shows the clinical characteristics according to *apo E* genotypes of the present subjects. The levels of total cholesterol were lower in E2/E3 and E3/E3 genotypes than in the rest of the genotypes. The levels of triglyceride had the highest value in the E3/E4 genotype. The frequency of diabetes was higher in the E2/E4, E3/E4, and E4/E4 genotypes than in the E2/E3 and E3/E3. The rest of the variables (i.e., percentage of obesity and smoking) showed no significant differences among genotypes.

4. Association between the frequencies of *apo E* genotypes and ICVD

The frequencies of *apo E* alleles with ICVD were as follows: E2, 22 (9.6%); E3, 186 (80.9%); and E4, 22 (9.6%). This was significantly different from the distribution in control subjects: E2, 18 (6.9%); E3, 201

Table 1. Clinical Characteristics of ICVD Patients (n=121)

Characteristics	Mean S.D.
Age (year)	48.1±22.2
Sex (m:f, %)	44:56
Total cholesterol (mg/dl)	187.5±47.0
HDL cholesterol (mg/dl)	46.8±12.6
Triglyceride (mg/dl)	134.9±83.8
Diabetes, n (%)	25(26.3)
Obesity, n (%)	13(16.9)
Ischemic heart disease, n (%)	27(28.4)

Table 2. Characteristics of ICVD Patients (n=121) According to the *apo E* Genotypes

Characteristics	E2/ E3	E2/ E4	E3/ E3	E3/ E4	E4/ E4
Age (year)	53.3±22.4*	53.3±17.4	51.4±21.7	40.6±20.7	38.7±24.8
Sex (m:f)	6:11	0:5	38:38	7:8	0:1
Total cholesterol (mg/dl)	171.0±50.8	207.8±43.8	190.0±49.4	205.6±28.4	207.0
HDL cholesterol (mg/dl)	50.1±10.5	50.2± 7.9	46.5±12.6	43.6±6.9	43.0
Triglyceride (mg/dl)	126.2±47.6	106.6±20.7	136.1±79.8	184.1±107.7	146.0
Diabetes, n (%)	3(21.4)	2(50.0)	14(23.0)	6(50.0)	1(100)
Obesity, n (%)	3(25.0)	1(25.0)	7(14.6)	2(28.6)	0(0)
Smoking, n (%)	3(21.4)	1(25.0)	22(37.9)	3(25.0)	0(0)

* Mean ± S.D.

(77.3%); and E4, 41 (15.8%). In addition, the distribution of *apo E* genotype in 121 patients with ICVD were as follows: E2/E3, 17 (14.8%); E2/E4, 5 (4.3%) E3/E3, 77 (67.0%); E3/E4, 15 (13.0%); and E4/E4, 1 (0.9%), which was a little different from the distribution in 132 control subjects: E2/E3, 14 (10.8%); E2/E4, 4 (3.1%) E3/E3, 78 (60.0%); E3/E4, 31 (23.8%); and E4/E4, 3 (2.3%).

The frequencies of E2/E3 and E3/E3 were higher in ICVD patients than in control groups (controls vs. patients: E2/E3, 10.8% vs. 14.8%; E3/E3, 60.0% vs. 67.0%), but the difference was not statistically significant ($p>0.05$). Especially, the frequencies of E3/E4 were lower in ICVD patients than those of in control groups (controls vs. patients: 23.8% vs. 13.0%). This difference was statistically significant ($p<0.05$) (Table 3). These results indicate that the E2 allele may be implicated as a risk factor for ICVD, whereas the E4 allele may be a protective factor against ICVD.

Discussion

ICVD is a multifactorial disease caused by the interactions of several genetic and environmental factors, including such recognized risk factors as high blood pressure, smoking, diabetes, obesity and advanced age. I examined the relationship between polymorphic genetic factor and ICVD. Apolipoprotein E is a polymorphic glycoprotein that plays a critical role

in cholesterol transport. *Apo E* polymorphisms have been extensively examined as a risk factor of vascular disease, including coronary artery disease (CAD)¹¹⁻¹⁶. However, studies concerning the relationship between gene polymorphisms potentially implicated and vascular diseases are leading to conflicting findings, due in part, to the difference in ethnic backgrounds between populations.

These led me to evaluate the impact of polymorphisms in the *apo E* gene on ICVD in individuals from Korea.

As a result, the frequencies of E2/E3 and E3/E3 genotypes were higher in ICVD patients than in the control group. In contrast, the frequencies of E3/E4 were lower in ICVD patients than in the control group (controls vs. patients: 23.8% vs. 13.0%) and the difference was statistically significant ($p<0.05$). These data are consistent with the report showing that the frequency of E2/E3 genotype was higher in ICVD patients than those of in control group (10.1% for patients, 1.4% for controls)⁷. On the other hand, my data is inconsistent with the study reporting the protective effect of E3⁷. To date, the *apo E2* allele has been reported to be associated with ICVD, whereas the *apo E4* allele was associated not only with ICVD^{11,17,18} but also with large-vessel ICVD¹⁹. Conversely, *apo E* was shown to be unrelated to cerebral infarction in Western populations^{14,20,21} and to cerebral infarction in the Japanese population²².

In conclusion, I examined the distribution of *apoE* genotypes in the Korean population and the association of *apo E* polymorphisms with ICVD, and I suggest that *apo E2* is a risk factor for ICVD, whereas E4 is a protective factor.

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Table 3. Frequency of *apo E* Genotype in Patients (n=115) and Control Subjects (n=130)

Genotypes	Controls n=130(%)	ICVD n=115(%)	<i>p</i> value
E2/E3	14(10.8)	17(14.8)	.346
E3/E4	4(3.1)	5(4.3)	.598
E3/E3	78(60.0)	77(67.0)	.260
E3/E4*	31(23.8)	15(13.0)	.031
E4/E4	3(2.3)	1(0.9)	.375

Statistical tests by χ^2 -test or Fisher's exact test (2-tailed).
* $p<0.05$

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