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Association among apolipoprotein E gene polymorphism, diabetes mellitus, and ischemic cerebrovascular disease in Koreans

Jae-Young Um[1,5,*], Do-Hwan Kim[2], Kwang-Ho Cho[2,3], Byung-Soon Moon[3], Mu-Song Son[1], Sejin Jeong[4], Hye-Jung Lee[5], Seung-Heon Hong[6] and Hyung-Min Kim[1]

[1]Department of Pharmacology, College of Oriental Medicine, Institute of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul, 130-701, Republic of Korea; [2]Department of Neurology, Wonkwang University School of Medicine, Iksan, Jeonbuk, 570-749, Republic of Korea; [3]Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk, 570-749, Republic of Korea; [4]Basic Institute of Natural Science, Wonkwang University Iksan, Jeonbuk, 570-749, Republic of Korea; [5]Acupuncture and Meridian Science Research Center, Kyung Hee University, Yongin, Gyeonggi 446-701, Republic of Korea; [6]College of Pharmacy, Wonkwang University, Iksan, Jeonbuk 570-749, Republic of Korea

SUMMARY

The association between apolipoprotein E (apoE) gene polymorphism and ischemic cerebrovascular disease (ICVD) has been controversial. These controversies may be due to inaccurate classification of patients and ethnic differences. The aim of the present study was to assess the relationship between apoE gene polymorphism and the development of ICVD in a population from Korea. We investigated 136 patients with ICVD and 357 controls without ICVD. No differences in the apoE genotypes frequencies (χ^2 = 3.660, *df* = 5, *P* = 0.454) and even in the alleles frequencies (χ^2 = 1.946, *df* = 2, *P* = 0.378) were observed in the ICVD patients compared with that in controls. The data have been compared with data found in other population groups. However, the risk of ICVD associated with apoE ε3/ε4 genotype was increased nearly 3-fold in subjects possessing the history of diabetes mellitus (OR 3.3, 95% CI 1.2-9.4, *P* = 0.026). We concluded that the apoE polymorphism is not associated with ICVD at least in the Korean population, but the apoE frequencies found in this study differ significantly from those obtained in Japanese.

Key words: Ischemic cerebrovascular disease; Apolipoprotein E; Polymorphism; Koreans

INTRODUCTION

Apolipoprotein E (apoE) is a 299 amino-acid protein with a central role in cholesterol transport and lipoprotein metabolism. The gene for apoE 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, ε4, ε3, ε2 which code for three major isoforms in plasma designated apo E4, apo E3, and apo E2 respectively, resulting in six common genotypes (Siest *et al*., 1995).

In addition, apoE is a key protein modulating the highly atherogenic apoB containing lipoproteins (Davignon *et al*., 1988) and is a candidate gene for the development of coronary artery disease (CAD).

*Correspondence: Jae-Young Um, Department of Pharmacology, College of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul, 130-701, Republic of Korea. Tel: +82 2 961 9262; Fax +82 2 967 7707; E-mail: jyum@khu.ac.kr

The ε2/ε2 genotype was the first to be implicated in premature coronary artery disease (Davignon *et al.*, 1988), which resulted in this polymorphism being extensively studied. These studies have not shown any clear relationship with the apoE polymorphism and risk of CAD, although in some there was a positive association (Lehtinen *et al.*, 1995; Stengard *et al.*, 1995; Lin *et a*l., 2004) yet in others no relationship (Luc *et al.*, 1994; Marshall *et al.*, 1994; Frikke-Schmidt *et al.*, 2001; Cerrato *et al.*, 2005). Similarly the evidence supporting a role for the apoE gene polymorphism as a risk factor for stroke is contradictory (Couderc *et al.*, 1993). These controversies may be due to inaccurate classification of subjects and ethnic differences. Therefore, the aim of this study was to compare the prevalence of the three most frequent alleles of apoE in a defined group of ischemic cerebrovascular disease (ICVD) patients with those in a control group in a Korean population.

MATERIALS AND METHODS

Subjects

One hundred and thirty-six patients (mean age 48.1 years) with ICVD during acute stage were identified according to well-defined criteria that included computerized tomography scanning, magnetic resonance imaging (MRI), and clinical signs (hemiparesis, hemiplegia, slurred speech, facial palsy, and so forth) from Wonkwang University Hospital in Iksan City, which is located in the Republic of Korea. Patients younger than 30 and older than 80 years of age were excluded. The control group consisted of 357 individuals (mean age 46.1 years) undergoing routine health screening. None of the controls had a history of ICVD. The control group were randomly recruited and matched with study patients for age and gender. All cases and controls (all Korean) gave informed consent before participating in the research protocol, which was approved by the ethics committee of the hospital.

Determination of apoE genotypes

The blood was stored at -20°C until genomic DNA was extracted, using an inorganic procedure (Miller *et al.*, 1988). The concentration of DNA was estimated by absorbance at 260 nm. The apoE polymorphism was detected by PCR amplification (Hixson and Vernier, 1990). Briefly a PCR reaction was carried out in a 20 μl volume containing 200 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM of each dNTP, and 1 U of r*Taq* DNA polymerase (Takara, Japan), with 1 μM of apoE F4/F6 (Bioneer, Republic of Korea). The primer pairs for each gene were as follows; F4: 5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′, F6: 5′-TAAGCTTGGCACGGCTGTCCAAGGA-3′ (Emi *et al.*, 1988). Amplification conditions were 5 min preincubation step at 95°C, 40 cycles of denaturation at 94°C for 40 s, annealing at 67°C for 40 s, and extension at 72°C for 40 s. A final extension for 10 min at 72°C was included (MJ Research). The PCR product was digested for 16h at 37°C with 5.5 units *Hha* I in the presence of 2 μg Bovine Serum Albumin. PCR products were then separated electrophoretically through 8% polyacrylamide gel with a pGEM DNA marker (Promega, USA) and the products visualized by ethidium bromide staining. The following fragments were obtained after restriction enzyme digestion: apoε2: 91, 81, 21, 18, 16, apoε3: 91, 48, 21, 18, 16, apoε4: 72, 48, 33, 21, 19, 18, 16. DNA of a subject with known apo ε2/ε2 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.

Statistical analysis

The mean levels of all numerical values were tested by the Student *t* test or ANOVA test. Comparisons of the allele frequencies of the apoE genotypes between the control and ICVD patients were carried out using the Pearson chi-square test. Odds ratios (OR) with 95% confidence intervals

(CI) were estimated by Mantel-Haenszel method. All statistical analyses were performed using SPSS v12.00 (SPSS Inc.) statistical analysis software. A P-value < 0.05 was considered statistically significant.

RESULTS

Clinical characteristics of subjects

Table 1 shows the clinical characteristics of the present subjects. A total of 136 patients were included in the analysis: 71 males (52.5%), mean age 48.1 ± 22.2, and range 30 - 80. There was no difference in average age and sex ratio between ICVD patients and controls. The frequencies of personal histories of hypertension, diabetes mellitus, and atrial fibrillation were significantly higher in ICVD patients (P < 0.001).

Association between apoE polymorphism and ICVD

Table 2 shows the clinical characteristics according to apoE genotypes in ICVD patients. The triglyceride level in subjects possessing the apoE ε3/ε4 genotype was higher than that of subjects with the apoE ε3/ε3 genotype, but the statistical power was very weak (P = 0.062). The levels of total cholesterol and HDL cholesterol showed no significant differences by apoE genotypes and even in compared with apoE ε3/ε3.

The genotype distribution in ICVD patients and controls did not deviate significantly from Hardy-Weinberg equilibrium. The distribution of apoE genotype in 136 patients with ICVD were as follows: ε2/ε2, 0 (0%); ε2/ε3, 19 (14.0%); ε2/ε4, 6 (4.4%); ε3/ε3, 86 (63.2%); ε3/ε4, 18 (13.2%); and ε4/ε4, 7 (5.1%), which was not different from the distribution in 357 control subjects: ε2/ε3, 52

Table 1. Comparisons of clinical characteristics between ICVD patients (n = 136) and controls (n = 357)

Characteristics	Controls	ICVD patients	OR (95% CI)[a]
Age, y	46.1 ± 32.5	48.1 ± 22.2	
Sex, % male	51.0	52.5	
Total cholesterol, mg/dl	177.5 ± 55.2	187.5 ± 47.0	
HDL cholesterol, mg/dl	54.8 ± 22.2	46.8 ± 12.6	
Triglyceride, mg/dl	107.5 ± 72.2	134.9 ± 83.8	
Diabetes mellitus, n (%)	47 (13.2)	38 (27.9)	2.6 (1.6 - 4.2)
Hypertension, n (%)	112 (31.4)	84 (61.8)	3.5 (2.3 - 5.3)
Atrial fibrillation, n (%)	37 (10.4)	40 (29.4)	3.6 (2.2 - 6.0)

Values are means ± S.D. [a]Mantel-Haenszel method was used to calculate the adjusted ORs with 95% CIs (Controls vs. ICVD patients).

Table 2. Clinical characteristics according to apoE genotypes in ICVD patients (n = 136)

Characteristics	Genotypes				
	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
ICVD patients, n (%)	19 (14.0)	6 (4.4)	86 (63.2)	18 (13.2)	7 (5.1)
Total cholesterol, mg/dl	171.0 ± 50.8	207.8 ± 43.8	190.0 ± 49.4	205.6 ± 28.4	207.0 ± 43.8
HDL cholesterol, mg/dl	50.1 ± 10.5	50.2 ± 7.9	46.5 ± 12.6	43.6 ± 6.9	43.0 ± 12.6
Triglyceride, mg/dl	126.2 ± 47.6	106.6 ± 20.7	136.1 ± 79.8	184.1 ± 107.7*	146.0 ± 80.8
Diabetes mellitus, %	21.1	50.0	23.3	50.0	0
Hypertension, %	42.1	66.7	64.0	66.7	71.4
Atrial fibrillation, %	31.6	16.7	32.6	16.7	28.6

Values are means ± S.D. *P = 0.062; By Student's t-test. ApoE ε3/ε3 was used as the reference in these analyses.

Table 3. OR (95% CI)[a] for apoE genotypes according to clinical characteristics in ICVD patients

Characteristics	n	Genotypes			
		ε2/ε3	ε2/ε4	ε3/ε4	ε4/ε4
ICVD patients	136	1.0 (0.6 - 1.8)	1.1 (0.4 - 2.9)	1.0 (0.6 - 1.9)	2.7 (0.9 - 8.0)*
Diabetes mellitus	38	0.9 (0.3 - 3.0)	3.3 (0.6 - 17.6)	3.3 (1.2 - 9.4)**	-
Hypertension	84	0.4 (0.1 - 1.1)	1.1 (0.2 - 6.5)	1.1 (0.4 - 3.3)	1.4 (0.3 - 7.7)
Atrial fibrillation	40	1.0 (0.3 - 2.8)	0.4 (0.0 - 3.7)	0.4 (0.1 - 1.5)	0.8 (0.1 - 4.5)

– Indicates no cases. [a]Mantel-Haenszel method was used to calculate the adjusted ORs with 95% CIs. ApoE ε3/ε3 was used as the reference in these analyses. *P < 0.06; **P < 0.05.

(14.6%); ε2/ε4, 15 (4.2%); ε3/ε3, 235 (65.8%); ε3/ε4, 48 (13.4%); and ε4/ε4, 7 (2.0%) (χ^2 = 3.660, df = 5, P = 0.454) (data not shown).

The results of logistic regression analyses are presented in Table 3. Compared with ε3/ε3 genotype subjects, the association between ICVD and apo ε4/ε4 genotype was observed, although the statistical power was very weak (OR 2.7, CI 0.9-8.0, P = 0.058). ORs associated with the other genotypes were not significant. Also, in subjects possessing the history of diabetes mellitus, the risk of ICVD associated with apoE ε3/ε4 genotype was increased nearly 3-fold (OR 3.3, 95% CI 1.2-9.4, P = 0.026). There were no differences in apoE genotypes when patients were stratified for the history of hypertenstion or atrial fibrillation.

Table 4 shows the association between apoE allelic frequencies and ICVD. The apoE allelic frequencies of the individuals with ICVD were as follows: ε2, 25 (9.2%); ε3, 209 (76.8%); and ε4, 22 (14.0%). It was not significantly different from the distribution in control subjects: ε2, 67 (9.4%); ε3, 570 (79.8%); and ε4, 77 (10.8%) (χ^2 = 1.946, df = 2, P = 0.378). Also, ε2 and ε4 carriers had no significant association with ICVD compared with ε3/ε3 genotype subjects.

Table 4. Allele frequencies of apoE in ICVD patients (n = 136) and controls (n = 357)

	Alleles		
	ε2	ε3	ε4
ICVD patients	0.09	0.77	0.14
Controls	0.09	0.80	0.11
Statistics[a]			
ε2 vs. ε3 vs. ε4	χ^2 = 1.946, df = 2, P = 0.378		
ε4 vs. ε2 & ε3	OR 1.343, CI = 0.886 -2.037, P = 0.165		
ε3 vs. ε2 & ε4	OR 1.193, CI = 0.853-1.669, P = 0.303		

[a]Statistical tests by χ^2 test (2-sided) or Mantel-Haenszel method.

DISCUSSION

Apo E is a polymorphic glycoprotein that plays a critical role in cholesterol transport. ApoE polymorphism has been extensively examined as a risk factor of vascular disease, including coronary artery disease (Snowden *et al*., 1991; Pedro-Botet *et al*., 1992; Saunders and Roses, 1993; Couderc *et al*., 1994; Coria *et al*., 1995; Kuusisto *et al*., 1995; Frikke-Schmidt *et al*., 2001; Lin *et al*., 2004; Cerrato *et al*., 2005). However, studies concerning the relationship between gene polymorphisms potentially implicated vascular diseases are leading to conflicting findings, due in part, to the difference in ethnic background between populations. These led us to evaluate the impact of polymorphisms in apoE gene on ICVD in individuals from Korea. In this study herein, there was no difference between ICVD patients and control subjects, indicating that apoE genotype was not a risk factor for ICVD in a Korean population. However, we found that diabetes mellitus enhances the relative risk for ICVD in subjects with the apoE ε3/ε4 genotype in Koreans.

Clinical and postmortem studies have demonstrated a close relationship between the apoE ε4 allele and the occurrence of myocardial infarction and coronary atherosclerosis (Hixon, 1991; Kosunen

et al., 1995; Wang *et al.*, 1995). In patients with cerebrovascular disease, only a limited number of studies have been performed, and these have produced mainly contradictory results (Pedro-Botet *et al.*, 1992; Couderc *et al.*, 1993; Kuusisto *et al.*, 1995; Nakata *et al.*, 1997; McCarron *et al.*, 1999; Catto *et al.*, 2000; Frikke-Schmidt *et al.*, 2001; MacLeod *et al.*, 2001; Lin *et al.*, 2004; Cerrato *et al.*, 2005). Different ethnic groups can also affect the results of these studies (Odawara *et al.*, 1996). The apoE ε2 allelic frequency of our Korean controls was higher than that in Japanese controls (0.09 vs. 0.05) (Zaman *et al.*, 1997; Kokubo *et al.*, 2000) and Europeans (0.09 vs. 0.06) (Brega *et al.*, 1998; Kowalska *et al.*, 1998; Kumar *et al.*, 2002), but similar to Taiwanese (0.09 vs. 0.08) (Wu *et al.*, 2002). In contrast, the apoE ε4 allelic frequency of our controls was close to that in Japanese controls (0.11 vs. 0.11) (Zaman *et al.*, 1997; Kokubo *et al.*, 2000), and higher than in Taiwanese (0.11 vs. 0.08) (Wu *et al.*, 2002). Even among Europeans there are geographic differences, with an ε4 frequency as high as 0.20 in Norway (Kumar *et al.*, 2002) and as low as 0.07 in Turkey (Brega *et al.*, 1998). Theses indicate that ethnic difference should be carefully considered in the studies on the association between apoE genotype and disease aetiology.

Although the statistical power was very weak, the association between ICVD and apo ε4/ε4 genotype was observed as it was compared with apoE ε3/ε3 (OR 2.7, CI 0.9-8.0, P = 0.058). Therefore, it is not possible from this study to completely exclude a role for apoE in the pathogenesis of ICVD. Further studies are necessary to clarify the association of this polymorphism or other functional polymorphisms closely linked to this polymorphism with the development of ICVD.

Diabetes is associated with atherosclerosis of the cerebral arteries (Kameyama *et al.*, 1994) and leads to important cerebral vascular changes that cause a decrease in cerebral blood flow (Mankovsky *et al.*, 1996). Our data show that association between diabetes and ICVD is particularly strong among carriers of the apoE ε3/ε4 genotype (OR 3.3, 95% CI 1.2-9.4, P = 0.026) (Table 3). This finding is in consistence with those previously published (Peila *et al.*, 2002). In the previous study, individuals with both type 2 diabetes and the apoE ε4 allele had an OR of 5.5 (CI 2.2 - 13.7) for Alzheimer's disease compared with those with neither risk factor.

In conclusion, the present results suggest that apoE polymorphism is less effective than the other susceptible genes and the environmental factors in the development of ICVD in Koreans.

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