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Relation of apolipoprotein E polymorphism to clinically diagnosed Alzheimer's disease in the Korean population

HEE-CHEOL KIM, MD, MS,[1] DAE-KWANG KIM, MD, PhD,[2] IN-JANG CHOI, PhD,[2] KYUNG-HEE KANG, MS,[2] SANG-DO YI, MD, PhD,[3] JONGHAN PARK, MD, MA, DMSc[4] AND YOUNG-NAM PARK, MD, PhD[1]

Departments of [1]Psychiatry, [2]Anatomy, [3]Neurology, Institute for Medical Genetics, Keimyung University School of Medicine and [4]Department of Psychiatry, Catholic University of Taegu-Hyosung School of Medicine, Taegu, Korea

Abstract The gene for human apolipoprotein E (APOE) is found on the long arm of chromosome 19 (19q13.2) and exists in three common allelic forms, ε2, ε3, and ε4. The APOE ε4 allele is overrepresented in Alzheimer's disease (AD) and is accepted as a genetic risk factor. Some studies reported a protective effect of the APOE ε2 allele for AD. However, there are some ethnic variations in the proportion of different APOE alleles and their relationship to AD. We examine the distribution of APOE alleles from 30 AD patients and 158 controls in Korea. The control subjects were all cognitively intact unrelated Koreans. The frequencies of APOE alleles in AD patients were 18.3% (ε2), 58.3% (ε3), and 23.3% (ε4). The corresponding frequencies in controls were 13.3% (ε2), 72.5% (ε3), and 14.2% (ε4). The frequency of the APOE ε2 allele in AD patients was not significantly different from that in controls. When statistical analysis was conducted after the exclusion of the APOE ε2 allele, the frequency of the APOE ε4 allele in AD patients was significantly higher than that in controls ($P < 0.05$). These results support that the APOE ε4 allele plays a role as a risk factor for AD in Koreans and suggest that the APOE ε2 allele may not play a protective role in the development of AD in Koreans.

Key words Alzheimer's disease, apolipoprotein E, genotype, Koreans, polymerase chain reaction.

INTRODUCTION

Apolipoprotein E (apoE = protein, APOE = gene) is a plasma protein involved in cholesterol transport and metabolism.[1] In humans, the brain is the most important site of apoE expression, after the liver.[2] ApoE is synthesized and secreted by glial cells, predominantly astrocytes.[3,4] ApoE is believed to play an important role not only in reactive synaptogenesis by delivering lipids to remodeling and sprouting neurons in response to tissue injury but also in physiological ongoing synaptic plasticity and maintenance of neuronal integrity as well as in cholinergic activity.[5–7] The gene for human apoE is found on the long arm of chromosome 19 (19q13.2) and exists in three allelic forms, designated as ε2, ε3, and ε4. Population studies have indicated that the APOE ε2 allele is associated with lower plasma low-density lipoprotein (LDL)–cholesterol levels and that the APOE ε4 allele is associated with higher plasma LDL–cholesterol levels, while the APOE ε3 allele is the most common allele.[8] The proportion of different APOE alleles varies between racial and ethnic groups, particularly with regard to the relative proportions of APOE ε2 allele and APOE ε4 allele.[9–12]

The APOE ε4 allele has been reported as a risk factor for early-onset and late-onset Alzheimer's disease (AD) in both familial and sporadic cases.[13,14] Inheritance of one or two APOE ε4 alleles is associated with younger age of onset in AD and the risk is related to a dose of APOE ε4 allele. The APOE ε4–AD association is strongest in Japanese subjects, followed by Caucasians, and is seemingly weaker among African Americans and Hispanics.[15] In

Correspondence: Hee-Cheol Kim, MD, MS, Department of Psychiatry, Keimyung University School of Medicine, 194, Dongsan-Dong, Joong-Gu, Taegu, 700–712, Korea. Email: mdhck@dsmc.or.kr

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contrast, a protective effect of the APOE ε2 allele for AD has been reported.[14,16] However, a Dutch population-based study presented that the APOE ε2 allele was associated with an increased risk of early-onset AD and a reduced survival.[17] In a sample of Italian subjects, the association between APOE ε2 allele and sporadic AD and early-onset AD was reported.[18] Therefore, it is likely that there are some ethnic variations in the proportion of different APOE alleles and their relationship to AD. In the present study, we examined the distribution of APOE alleles from 30 AD patients and 158 controls in Korea.

MATERIALS AND METHODS

Subjects

Blood for genomic DNA was obtained from 30 AD patients and 158 cognitively intact controls. The AD patients were recruited from the patients who consecutively visited the psychiatric department of two university hospitals or admitted to the institution for patients with dementia in Taegu, Korea. The diagnosis for dementia was made according to DSM-IV criteria[19] and the diagnosis for probable AD was made according to NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association) criteria.[20] The mean age of AD patients was 71 years and the age range 60–88 years. The 158 control subjects were all cognitively intact unrelated Koreans. The mean age of control subjects was 43 years and the age range 20–73 years.

Amplification of APOE sequences

Peripheral blood DNA was isolated using a modified method of Maniatis *et al*.[21] Extracted DNA was amplified by polymerase chain reaction (PCR) in a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CT, USA) using primer 1 (5′-ACAGAATTCGCCCCG-GCCTGGTACAC-3′) and primer 2 (5′-TAAGCTTG-GCACGGCTGTCCAAGGA-3′) described by Emi *et al*.[22] The PCR mixture contained 300 ng of genomic DNA, 5 μL of 10× reaction buffer (100 mmol/L Tris HCl, pH 8.3, 500 mmol/L KCl, and 25 mmol/L $MgCl_2$), 5 pmol of each primer 1 and primer 2, 200 μmol/L of each dNTPs, and 0.5 unit *Taq* DNA polymerase (PE Applied Biosystems, Foster City, CA, USA) in a final volume of 50 μL. Polymerase chain reaction was performed for one cycle of denaturation at 94°C for 10 min and 30 cycles of denaturation at 94°C, annealing at 60°C, and extension 70°C for 30 s, respectively. A final extension step of 70°C for 7 min was done. The PCR products were identified by 3% metaphor agarose (FMC) and visualized by ethidium bromide staining.

Restriction isotyping of amplified APOE sequences

For restriction isotyping of APOE alleles 20 μL of the PCR product was digested with two units of *Hha*I for 10 h. The digested fragments were electrophoresed on 5% NuSieve 3 : 1 agarose (FMC) at 100 V for 30 min. After electrophoresis, the gel was stained with ethidium bromide and the size of *Hha*I digested fragments was estimated by comparison with 20 base pair DNA ladder size marker (FMC) (Fig. 1).

Statistical analysis

We estimated allelic and genotypic frequencies of the APOE for the AD patients and controls by counting alleles and genotypes and calculating sample proportions. Comparisons of allele frequencies and genotype frequencies of APOE were made using chi-squared analysis.

RESULTS

The genotype frequencies are shown in Table 1. The most frequent genotype for all subjects was ε3/ε3 (AD subjects, 36.7%; control subjects, 58.2%). The frequencies of APOE alleles in AD patients were 18.3% (ε2), 58.3% (ε3), and 23.3% (ε4). The corresponding frequencies in controls were 13.3% (ε2), 72.5% (ε3), and 14.2% (ε4). The frequency of the APOE ε2 allele in AD patients was not significantly different from that in controls. When statistical analysis was conducted after the exclusion of the APOE ε2 allele, the frequency of the APOE ε4 allele in AD patients was significantly higher than that in controls ($\chi^2 = 4.11$, d.f. = 1, $P < 0.05$).

DISCUSSION

The main object of the present study was to examine the distribution of the APOE alleles in Korean AD patients. In contrast with previous reports, our results represented relative higher frequency of the APOE ε2 allele in AD patients and controls. In particular, the frequency of the APOE ε2 allele in our AD patients was much higher than previous reports in other racial groups including Caucasians, Hispanics, African Americans, and Japanese.[15] The frequency of the APOE ε3 allele in our AD patients was similar to that in Caucasians and African Americans, but lower than that in Hispanics and Japanese.[15] The frequency

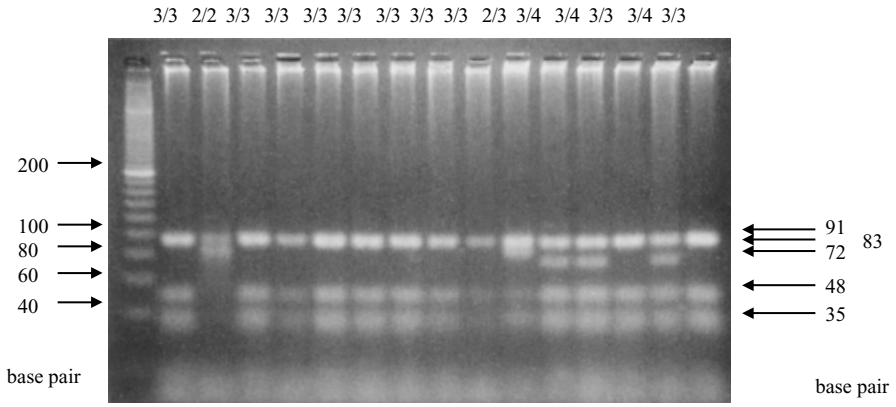

Figure 1. Example of APOE alleles from 15 different people. The three major alleles of APOE differ by single nucleotide substitutions within the amino acid codons at positions 112 (Cys→Arg; ε3→ε4) and 158 (Arg→Cys; ε3→ε2). These sequence differences can be demonstrated by using polymerase chain reaction to amplify DNA obtained from blood samples. In restriction isotyping of APOE, the sizes of *Hha*I digested fragments in each isoform are visualized by UV illumination. The APOE ε2 allele was characterized by the presence of the 83 and 91 base pair bands, the APOE ε3 allele by the 35, 48, and 91 base pair bands, and the APOE ε4 allele by the 19, 35, 48, and 72 base pair bands. Each of the heterozygous types is shown by mixed bands of homozygotes.

of the APOE ε4 allele in our AD patients was lower than that in Caucasians, African Americans, and Japanese, but slightly higher than that in Hispanics.[15] Compared with controls, the frequency of the APOE ε2 allele in our AD patients was not significantly different from that in controls. The frequency of the APOE ε4 allele in our AD patients was 23.3%, which was significantly higher than that in controls. These results do not support that the APOE ε2 allele protects the development of AD, but do support that the APOE ε4 allele plays a role as a risk factor for AD.

According to previous studies in Koreans,[23,24] the frequency of the APOE ε4 allele in AD patients was significantly higher than that in controls and the association between the APOE ε4 allele and AD was consistently reported. These results, including our data, confirm the importance of the APOE ε4 allele as a risk factor for AD in Koreans. In contrast with the APOE ε4 allele, the role of the APOE ε2 allele in the development of AD in Koreans was controversial. One study reported the APOE ε2 allele frequency (4.1%) in Korean AD group did not differ from that (4.6%) in the control group.[23] The other study reported the same frequency (10.0%) of the APOE ε2 alleles in Korean AD patients and controls which was a relatively high percentage compared with other racial groups' results.[24] Compared with the present study, the frequency of the APOE ε2 allele of controls in that study[24] was not significantly different from that in the present study ($\chi^2 = 1.30$, d.f. = 2, $P = 0.523$). However, the frequency of the APOE ε2 allele of controls in another Korean study[23] was significantly different from that in the present study ($\chi^2 = 25.79$, d.f. = 2, $P = 0.000$). The reason for this difference is not determined yet, however, probably due to differences in population characteristics. Therefore, there is a strong need to study large samples for further clarification. The frequency of the APOE ε2 allele in our Korean AD patients was higher than that in any other previous reports, but was not significantly different from that in controls. These results suggest that the APOE ε2 allele may not play a protective role in the development of AD in Koreans.

The association between the APOE ε4 allele and AD has been reported primarily from Caucasian

Table 1. APOE genotypes and allele frequencies

	By genotype						By allele		
	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4	ε2	ε3	ε4
AD ($n = 30$)	1	5	4	11	8	1	11	35	14
	3.3%	16.7%	13.3%	36.7%	26.7%	3.3%	18.3%	58.3%	23.3%
Controls ($n = 158$)	2	20	18	92	25	1	42	229	45
	1.3%	12.7%	11.4%	58.2%	15.8%	0.6%	13.3%	72.5%	14.2%

populations in the USA and Europe. However, the APOE has been extensively investigated in populations worldwide because of its role in lipid metabolism and ischemic cardiovascular disease. Nowadays, the emergence of the APOE ε4 allele as a major risk factor for AD has been confirmed in more than 100 studies worldwide. The association between the APOE ε4 allele and AD is strongest in Japanese subjects, followed by Caucasians, and is seemingly weaker among African Americans and Hispanics.[15] A meta-analysis of 40 studies representing nearly 30 000 APOE alleles concluded that the APOE ε4 allele represented a major risk factor for AD in Caucasians, African Americans, Hispanics, and Japanese, across all ages between 40 and 90 years, but that the effect diminished after age 70.[15]

The role of the APOE ε2 allele in the development of the AD is less clear. A protective effect of the APOE ε2 allele for AD has been reported.[14,16] Although a number of studies confirmed the lowered APOE ε2 allele frequency in AD patients versus controls, others did not find the association. One study reported a significant increase in the frequency of the APOE ε2 allele in Italian patients with sporadic AD as well as with familial early-onset AD.[18] An increased APOE ε2 allele frequency was also reported for AD patients of African-American descent, while a decreased APOE ε2 allele frequency was found in Caucasian AD patients.[25] Furthermore, a Dutch population-based study presented that the APOE ε2 allele was associated with an increased risk of early-onset AD and a reduced survival.[17] Our study also showed a tendency to increase in the frequency of the APOE ε2 allele in AD patients in spite of no significant difference in the frequency of the APOE ε2 allele between AD patients and controls. There are at least three possible explanations for these discrepancies between studies of the role of the APOE ε2 allele in AD patients.

The first explanation for the different allelic associations of the APOE among Koreans and other racial groups with AD is that the associations are due to linkage disequilibrium, rather than to a direct effect of the APOE alleles. The linkage disequilibrium implies that the risk allele of an unknown causal genomic polymorphism is always accompanied by the APOE allele, because APOE and the causal genomic polymorphism are close together on chromosome 19 and recombination during meiosis is unlikely. An AD susceptibility gene may be in the linkage disequilibrium with the APOE allele in AD patients. Recently, several new polymorphisms within the transcriptional regulatory region of the APOE gene were reported.[26,27] Bullido *et al*. identified a diallelic polymorphism in the promoter region of the APOE gene, –491 base pairs upstream of the APOE transcriptional start site (–491 A/T), and found that homozygosity for the –491 A allele was associated with AD, being independent of APOE ε4 allele status.[26] Additionally, they reported *in vitro* studies, which suggested that the –491 A/T polymorphism altered the level of APOE expression thereby modulating the risk for AD.[26] Because of the close physical distance between the –491 A/T and known APOE polymorphisms and the consistent association of the latter with AD, the association of the –491 A (or –491T) allele with AD reflects the strong linkage disequilibrium between the –491 A (or –491T) allele with the known APOE polymorphism. Recent study presented a stronger association between the APOE ε2 allele and –491T allele in the Dutch population ($P = 0.002$ in controls) than in the Spanish one ($P = 0.07$ in controls).[28] The second possibility is that genes other than APOE may confound studies. This explanation for the lack of consistency in the protective effect of APOE ε2 allele for AD is less likely to be the case in our study. The frequency of the APOE ε4 allele was consistently increased in our AD patients and the APOE ε4 allele played a role as a risk factor in the development of AD. Another explanation may be that mutations may have occurred in the rare APOE ε2 allele that leads to an increased risk of AD. The new APOE alleles resulting from such mutations may be important determinants of AD risk in some populations but not in others. Such a mechanism cannot be excluded unless the APOE gene is fully sequenced in all patients.

In interpreting the present results, some limitations should be noted. One limitation is that the controls of the present study had a wide range of age distribution and were not age matched. We did not have sufficient old aged controls to compare with AD patients. However, when we compared controls aged 60 years or more ($n = 31$) with controls aged less than 60 years ($n = 127$), the frequencies of three different APOE alleles were not significantly different between them ($\chi^2 = 4.18$, d.f. = 2, $P = 0.123$). Recent Japanese study also showed that there were no significant differences in the distribution of the APOE phenotypes between the younger (aged 65 years or under) and the older (aged more than 65 years) subgroups of the normal healthy controls.[29] Another limitation is that the number of AD patients was too small to generalize the study results. In spite of these limitations, it is noteworthy that this study shows the relative high frequency of the APOE ε2 allele in AD patients and shows no significant difference in the frequency of the APOE ε2 allele between AD patients and controls.

CONCLUSIONS

In the present study of Korean AD, we presented an increased frequency of the APOE ε4 allele in AD patients versus controls and presented no significant difference in the frequency of the APOE ε2 allele between AD patients and controls. These results support that the APOE ε4 allele plays a role as a risk factor for AD in Koreans and suggest that the APOE ε2 allele may not play a protective role in the development of AD in Koreans. Our results with Korean subjects warrant further study with large samples.

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