

Association between apolipoprotein E polymorphism and Alzheimer's disease in Koreans

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Abstract

We analyzed the apolipoprotein E (APOE) genotypes of 110 probable AD patients and 226 cognitively normal controls in Koreans. The APOE ϵ 4 allele was more prevalent in both early- and late-onset AD patients ($P < 0.01$) than in controls. The odds for the APOE ϵ 4-heterozygous subjects were 2.7 (95% CI = 1.6–4.5), and those for the APOE ϵ 4-homozygous subjects were 17.4 (95% CI = 2.0–147.3). But the odds were not uniform across age groups, and were higher in women than in men. Although the APOE ϵ 2 allele frequency did not differ by diagnosis, the patients carrying an APOE ϵ 2 allele showed delayed age-at-onset ($P = 0.02$). In conclusion, the APOE ϵ 4 allele increased the risk for AD in dose-dependent manner, and the APOE ϵ 4-conferred AD risk was age- and sex-dependent in Koreans. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Alzheimer's disease; Apolipoprotein E; Koreans; Risk; Age-at-onset; Age; Sex

The apolipoprotein E (APOE) ϵ 4 allele has been established as an important genetic susceptibility factor for Alzheimer's disease (AD) in numerous ethnic populations. The APOE ϵ 4 allele increases the risk for AD and lowers the age-at-onset of AD in dose-dependent manner [5]. But its contribution to the development of AD didn't seem to be equal in all ethnic groups [5], and the ethnic differences in the APOE allelic frequencies and the APOE ϵ 4-conferred AD risk were evident even among East Asians. The APOE ϵ 4 allele was more prevalent and the APOE ϵ 2 allele was less prevalent in Japanese than in Chinese [5,7,8,11]. The APOE allelic frequencies in Koreans [2] were also different from those in Japanese [5] and Chinese [4]. The APOE ϵ 4-AD association in Japanese was substantially stronger than that in Chinese [5,7,8,11]. The influence of the APOE ϵ 4

allele on the age-at-onset of AD was evident in Japanese [9,14], while it was not significant in Chinese [11].

Thus, we aimed to investigate the influence of the APOE polymorphism on the risk for AD and on the age-at-onset of AD in Koreans, determining the APOE genotypes in 110 probable AD patients and 226 cognitively normal controls.

All the AD patients and controls were unrelated Koreans. We administered standardized CERAD Clinical Assessment Battery [13] and Modified Hachinski Ischemic Score (MHIS) [6]. Following this evaluation, a consensus committee meeting was held to determine a diagnosis for each subject; diagnoses for dementia were according to DSM-IV criteria [1] and diagnoses for AD were according to NINCDS-ADRDA criteria [12]. The subjects who were diagnosed as cognitively normal at the consensus committee meeting and got less than four of MHIS were included in the normal control group.

Genomic DNA was extracted from venous blood, and APOE genotyping was done according to the method

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described by Wenham et al. [15]. Two hundred nanograms of genomic DNA was mixed with polymerase chain reaction (PCR) mixture composed of 20 pmol of each primer, 10 μ l 20% glycerol, 5 μ l 25 mM MgCl₂ and 5 μ l 10 \times PCR reaction buffer (Tris 100 mM, pH 8.3, KCl 500 mM), and amplified for 30 cycles at 65°C for 0.5 min, 72°C for 2 min and 94°C for 1 min.

We estimated allele and genotype frequencies of APOE for the controls and AD cases by counting alleles and genotypes and calculating sample proportions. Hardy–Weinberg equilibrium was tested by the likelihood-ratio test [10]. Comparisons of allele frequencies and genotype frequencies of APOE were made using Chi square analysis and Fisher's exact test when appropriate. Logistic regression models adjusting for age and sex were used to calculate the odds ratios (ORs) for AD.

AD patients were slightly older than controls (70.5 \pm 8.0 years versus 68.3 \pm 4.6 years, $P < 0.05$ by two-tailed Student's t -test). But the AD group and control group did not differ by gender. The AD group was comprised of 65 late-onset AD (LOAD) patients and 45 early-onset AD (EOAD) patients. The mean age-at-onset of the EOAD patients was 58.5 \pm 4.4 years and that of the LOAD patients was 72.8 \pm 4.9 years. The mean age of the EOAD patients was 62.7 \pm 4.6 years and that of the LOAD patients was 75.9 \pm 4.6 years. The EOAD group and LOAD group did not differ by gender or severity of impairments in cognitive functions and activities in daily life (data are not presented).

The distributions of the APOE genotypes of the AD group, EOAD group, LOAD group and control group are presented in Table 1. The distribution of the APOE genotypes in each group was in Hardy–Weinberg equilibrium. As expected, the APOE ϵ 4 allele frequency of the AD group was significantly higher than that of the control group

Table 1
The distributions of the APOE genotypes and alleles in AD patients and controls in Koreans^a

	AD			Control (%) (<i>n</i> = 226)
	Total (%) (<i>n</i> = 110)	EOAD (%) (<i>n</i> = 45)	LOAD (%) (<i>n</i> = 65)	
APOE genotypes				
ϵ 2/ ϵ 3	7.3	–	12.3	8.0
ϵ 2/ ϵ 4	0.9	–	1.5	1.3
ϵ 3/ ϵ 3	53.6	55.6	52.3	74.4
ϵ 3/ ϵ 4	32.7	35.6	30.8	15.9
ϵ 4/ ϵ 4	5.5	8.8	3.1	0.4
APOE alleles				
ϵ 2	4.1	–	6.9	4.6
ϵ 3	73.6	73.3	73.9	86.3
ϵ 4	22.3	26.7	19.2	9.1

^a The EOAD group is comprised of early-onset AD patients in whom AD developed before 65. The LOAD group is comprised of late-onset AD patients in whom AD developed at 65 or later. The distribution of the APOE genotypes in each group was in Hardy–Weinberg equilibrium.

($\chi^2 = 22.2$, d.f. = 1, $P < 0.01$) at the expense of the APOE ϵ 3 allele frequency ($\chi^2 = 16.1$, d.f. = 1, $P < 0.01$). The overexpression of the APOE ϵ 4 allele in the AD patients compared with the controls remained significant when we analyzed the LOAD patients ($\chi^2 = 10.4$, d.f. = 1, $P < 0.01$) and EOAD patients ($\chi^2 = 22.0$, d.f. = 1, $P < 0.01$) separately. We also investigated the relationship between the APOE ϵ 2 allele and the development of AD, because the role of the APOE ϵ 2 allele in the development of AD was not evident in East Asians [5]. In the present study, the APOE ϵ 2 allele frequency in the AD group did not differ from that in the control group ($\chi^2 = 0.1$, d.f. = 1, $P > 0.1$), indicating that the APOE ϵ 2 allele does not play a protective role in Koreans.

The age- and sex-adjusted OR for subjects with one or two copies of the APOE ϵ 4 alleles was 3.2 (95% CI = 1.9–5.4), which was comparable to those in Chinese [7,8,11] as well as in Caucasians [5], but was about a half of those in Japanese [5]. And OR for the APOE ϵ 4 heterozygous subjects was 2.7 (95% CI = 1.6–4.5) and that for the APOE ϵ 4 homozygous subjects was 17.4 (95% CI = 2.0–147.3), indicating that the APOE ϵ 4 allele increased the risk for AD in dose-dependent manner. The dose-dependent contribution of the APOE ϵ 4 allele remained significant when we analyzed the LOAD patients and the EOAD patients separately. The ORs for AD were higher in the EOAD patients than in the LOAD patients, which is in agreement with the results in Japanese [3,14] (Table 2).

Table 3 shows the frequency of the APOE alleles stratified by the age groups and sex. Age was stratified as four classes (50–64, 65–69, 70–74 and 75–89 years). There was no evidence for heterogeneity of the APOE ϵ 4 allele frequency among the age groups and sex in the controls. But, although an elevated frequency of the APOE ϵ 4 allele was detected in every age group of the AD patients, the risks for AD associated with the APOE ϵ 4 allele were not equal in all age groups. The ORs for AD increased between ages 50 and 69 years but declined with age thereafter, indicating that the APOE ϵ 4-conferred AD risk is age-dependent. And the age-adjusted ORs in women were higher than those in men, suggesting that the APOE ϵ 4-conferred AD risk may not be uniform across sexes.

Table 4 shows the distribution of the ages-at-onset by the status of APOE. The mean age-at-onset in the patients with one or two APOE ϵ 4 alleles was lower than that in the patients with no APOE ϵ 4 allele in both sexes, although the difference reached statistical significance only in men ($P = 0.03$ by Mann–Whitney U test). The patients carrying an APOE ϵ 2 allele showed significantly delayed ages-at-onset than those carrying no APOE ϵ 2 allele ($P = 0.02$ by Mann–Whitney U test). But the delay of age-at-onset was significant only in those carrying the APOE ϵ 2/ ϵ 3 genotype ($P = 0.01$ by Mann–Whitney U test), and the age-at-onset of the only one patient who carried the APOE ϵ 2/ ϵ 4 genotype was 65 years old.

As in other ethnic groups from East Asia, i.e. Chinese and

Table 2
Odds ratios for developing AD conferred to the APOE genotypes in Koreans

	OR (95% C.I.) ^a		
	AD	EOAD	LOAD
APOE genotype			
$\epsilon 3/\epsilon 3$	Referent group	Referent group	Referent group
$\epsilon 2/\epsilon 3$	1.4 (0.5–2.8)	– ^b	2.1 (0.6–7.5)
$\epsilon 2/\epsilon 4$	1.0 (0.1–9.8)	– ^b	2.8 (0.2–34.0)
$\epsilon 3/\epsilon 4$	2.9 (1.7–5.2)	3.1 (1.3–7.0)	3.1 (1.2–7.6)
$\epsilon 4/\epsilon 4$	24.7 (2.8–214.5)	20.6 (2.0–208.0)	35.9 (2.5–510.4)
APOE $\epsilon 4$ allele			
0 copy	Referent group	Referent group	Referent group
1 copy	2.7 (1.6–4.5)	3.0 (1.5–6.2)	2.4 (1.3–4.5)
1 or 2 copies	3.2 (1.9–5.4)	3.9 (1.8–8.5)	3.3 (1.4–7.5)
2 copies	17.4 (2.0–147.3)	34.5 (3.7–324.6)	8.5 (0.8–96.2)

^a ORs were calculated using standard logistic regression controlling age and sex. ^bUncalculable due to empty cell.

Japanese, the APOE $\epsilon 4$ allele conferred risk for AD in dose-dependent manner in Koreans, too. But the APOE $\epsilon 4$ -AD association in Koreans was much weaker than that in Japanese. Actually the odds of AD attributable to the APOE $\epsilon 4$ allele in Japanese were higher than those in all of the other ethnic groups [5,7,8,11]. Several possible factors to explain this strong APOE $\epsilon 4$ -AD association in Japanese can be addressed. First, the distribution of the ages of subjects may cause the difference in the risk for AD conferred to the APOE $\epsilon 4$ allele, since the patterns of changes in the risk for AD associated with the APOE $\epsilon 4$ allele were different among East Asians. In Japanese, ORs for AD associated with the APOE $\epsilon 4$ allele increased steadily between ages 40 and 60 years and then declined with age thereafter [5]. But in Chinese, ORs increased between ages 60 and 84 years and then declined with age thereafter [8]. In the present study,

ORs increased between ages 50 and 69 years and then declined with age thereafter. Thus the odds of AD would be higher if more subjects at high-risk age were included in the population studied. Second, gender difference between the AD patients and controls might have also contributed to the high ORs in Japanese. We note that the Japanese AD patients and the Japanese controls pooled by Farrer et al. [5] significantly differed by gender; the proportion of female was 70.5% in the AD group and 27.6% in the control group ($P < 0.01$ by χ^2 test). Considering that the OR for AD associated with the APOE $\epsilon 4$ allele was higher in women than in men in the present study and a meta-analysis by Farrer et al. [5], this big gender difference might have also contributed to the high ORs in Japanese, at least in part. Finally some unknown differences particularly present in Japanese might have contributed to the strong APOE $\epsilon 4$ -AD association. We

Table 3
The distributions of the APOE genotypes and alleles in AD patients and controls stratified by age groups and sex in Koreans

		Genotypic frequency (%)					Allelic frequency (%)			OR (95% CI)
		$\epsilon 2/\epsilon 3$	$\epsilon 2/\epsilon 4$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$	$\epsilon 4/\epsilon 4$	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	
Age group (years)										
50–64	AD (N = 29)	– ^a	– ^a	58.6	31.0	10.4	– ^a	74.1	25.9	3.3 (1.1–9.9)
	Control (N = 45)	8.9	2.2	73.3	15.6	– ^a	5.6	85.5	8.9	
65–69	AD (N = 20)	5.0	– ^a	35.0	50.0	10.0	2.5	62.5	35.0	7.0 (2.5–19.8)
	Control (N = 97)	9.3	– ^a	73.2	16.5	1.0	4.6	86.1	9.3	
70–74	AD (N = 24)	8.3	4.2	58.3	25.0	4.2	6.3	74.9	18.8	2.4 (0.8–7.0)
	Control (N = 64)	4.7	3.1	78.1	14.1	– ^a	3.9	87.5	8.6	
75–85	AD (N = 37)	13.5	– ^a	56.8	29.7	– ^a	6.8	78.3	14.9	1.4 (0.4–5.3)
	Control (N = 20)	10.0	– ^a	70.0	20.0	– ^a	5.0	85.0	10.0	
Sex group										
Women	AD (N = 87)	6.9	1.1	48.3	36.8	6.9	4.0	70.1	25.9	4.4 (2.4–8.0)
	Control (N = 182)	9.3	1.6	74.4	15.9	0.4	5.5	86.0	8.5	
Men	AD (N = 23)	8.7	– ^a	73.9	17.4	– ^a	4.3	87.0	8.7	0.6 (0.1–2.2)
	Control (N = 44)	2.3	– ^a	75.0	22.7	– ^a	1.1	87.5	11.4	
All	AD (N = 110)	7.3	0.9	53.6	32.7	5.5	4.1	73.6	22.3	3.19 (1.9–5.4)
	Control (N = 226)	8.0	1.3	74.4	15.9	0.4	4.6	86.3	9.1	

^a Uncalculable due to empty cell.

Table 4
Age-at-onset in the AD group according to the APOE genotypes

	Age-at-onset of AD					
	Total		Female		Male	
	N	Mean \pm SD	N	Mean \pm SD	N	Mean \pm SD
APOE genotype						
ϵ 2/ ϵ 3	8	74.0 \pm 4.4	6	73.3 \pm 4.3	2	76.0 \pm 5.7
ϵ 2/ ϵ 4	1	65	1	65	–	–
ϵ 3/ ϵ 3	59	67.1 \pm 9.0	42	68.2 \pm 8.6	17	64.2 \pm 9.9
ϵ 3/ ϵ 4	36	65.9 \pm 8.0	32	67.3 \pm 7.2	4	54.5 \pm 4.5
ϵ 4/ ϵ 4	6	62.8 \pm 4.9	6	62.8 \pm 4.9	–	–
Number of ϵ 4						
0	67	67.9 \pm 8.9	48	68.9 \pm 8.3	19	65.5 \pm 10.2
1	37	65.8 \pm 7.9	33	67.2 \pm 7.1	4	54.5 \pm 4.5
2	6	62.8 \pm 4.9	6	62.8 \pm 4.9	–	–
Number of ϵ 2						
0	101	66.4 \pm 8.5	80	67.4 \pm 7.9	21	62.4 \pm 9.9
1	9	73.0 \pm 5.1	7	72.1 \pm 5.0	2	76.0 \pm 5.7

also investigated whether the APOE ϵ 2 allele contributed to development of AD in Koreans, for its protective role against AD was not conclusive in Japanese or Chinese [5,11]. In the present study, since the frequencies of the APOE ϵ 2 did not differ by diagnosis, it is not conclusive that the APOE ϵ 2 allele is protective against the development of AD. However, since the frequencies of the APOE ϵ 2 in normal controls did not differ across the age groups ($P > 0.1$) and the APOE ϵ 2 carriers showed a trend of delayed age-at-onset of AD in both sexes, the APOE ϵ 2 allele could be associated with a delayed onset in Korean AD patients.

In conclusion, the APOE ϵ 4 allele increased the risk for AD in dose-dependent manner, and the APOE ϵ 4-conferred AD risk was age- and sex-dependent in Koreans.

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