Apolipoprotein E Polymorphism and Age at Onset of Alzheimer's Disease in a Quadriethnic Sample

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Key Words

Alzheimer's disease • Apolipoprotein E • Age at onset • Ethnicity

Abstract

Background: The relationship between the apolipoprotein E (ApoE) genotype and the risk for developing Alzheimer's disease (AD) or age at onset of AD is relatively well established in Caucasians, but less established in other ethnicities. We examined the association between the ApoE genotype and age at onset of AD in a quadriethnic group of community-dwelling AD patients. **Methods:** AD patients were evaluated at 2 university-based outpatient memory disorder clinics. The ethnic distribution was as follows: Caucasians (n = 1,083), Hispanics (n = 55), African Americans (n = 84) and Koreans (n = 87). All were diagnosed with probable AD according to NINCDS-ADRDA diagnostic criteria. **Results:** After adjusting for ethnicity, the $\epsilon 4$ allele was significantly associated with earlier age at onset ($p < 0.0001$) for the combined group. Within ethnic groups, the effect of Apo $\epsilon 4$ on age at onset was significant in Caucasians ($p < 0.0001$) and African Americans ($p < 0.05$), but nonsignificant in Koreans ($p = 0.43$) and in the smaller Hispanic ($p = 0.07$) group. **Conclusions:** The association between Apo $\epsilon 4$ and younger age at onset was

significant in Caucasians and African Americans, where the $\epsilon 4$ allele was also most frequent. This study suggests that the impact of ApoE polymorphism on age at onset of AD may be different among distinct ethnic groups.

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Introduction

Apolipoprotein E (ApoE) is a plasma protein important in the transport of lipids. The presence and gene dose of Apo $\epsilon 4$ was reported to be a risk factor for late onset Alzheimer's disease (AD) [1] and was also associated with early onset AD [2]. The relationship between the ApoE genotype and risk of AD is relatively well established in Caucasian populations, but is less clearly established in other racial/ethnic groups [3–7]. Apo $\epsilon 4$ may also be a modulator of age at onset of AD in a dose-dependent manner for several ethnic groups: Caucasian, Hispanic, African American and Japanese [3]. In contrast to Apo $\epsilon 4$, Apo $\epsilon 2$ has been hypothesized to be a protective factor against the development of AD [3, 8, 9]. Apo $\epsilon 3$ has been suggested to have a neutral effect or mildly beneficial effect on the risk of AD in a few small clinical [10, 11] and laboratory studies [12, 13], but clinical evidence from

larger studies is lacking. The current study examined the relationship between ApoE polymorphisms and age at onset of AD in 4 defined racial/ethnic groups of older adults with AD residing in the community: Caucasians, Hispanics, African Americans and Koreans.

Subjects and Methods

Patients

The participants for this study were recruited from 2 university-based memory clinic patient cohorts: Baylor College of Medicine, Department of Neurology, Alzheimer's Disease and Memory Disorders Center, Houston, Tex., USA, between 1989 and 2009; and Dementia Center of Daegu Catholic University Medical Center, Daegu, Korea, between 2003 and 2009. From the Alzheimer's Disease and Memory Disorders Center, 3 groups (Caucasians, Hispanics and African Americans) were identified and only Korean subjects were recruited from the center in Korea. All participants gave their written informed consent before participating in this study. The data collection protocol was approved by the Institutional Review Board of Baylor College of Medicine in Houston and the Institutional Review Board of Daegu Catholic University Medical Center in Daegu.

Methods

Diagnosis and Age at Onset

Diagnosis of dementia was made according to DSM-IV criteria [14] and that of probable AD was made according to NINCDS-ADRDA criteria [15]. All Baylor participants underwent an extensive evaluation as described elsewhere [16]. At both sites, physical and neurological examinations, laboratory tests, brain imaging study and neuropsychological tests, including the Mini-Mental State Examination (MMSE or K-MMSE) were administered.

Age at onset was estimated to the nearest half year by the clinician, using standardized methodology [17]. Briefly, this method included an interview with the patient and all available informants, review of medical records to look for previous chronologies, and asking the patient's caregiver to estimate the duration of 34 symptoms commonly associated with AD. The physician then estimated the age at onset to the nearest half year after resolving any discrepant information through further questioning, and by relating hypothesized time frames to the patient's life events.

ApoE Genotyping

The ApoE genotype was determined from serum by the method described by Hixson and Vernier [18]. Genomic DNA was prepared from peripheral leucocytes. Restriction isotyping was performed by polymerase chain reaction (PCR) amplification of ApoE DNA sequences containing amino acids at the 112 and 158 positions in a DNA thermal regulator using the oligonucleotide primers F4 and F6. The amplified products were then digested with HhaI and subjected to electrophoresis on polyacrylamide gels. The pattern of migration of digested fragments indicated the ApoE genotype. Although genotyping was not done in the same laboratory, the method used is both standardized and published, as well as widely used.

Statistical Analysis

Clinical and demographic variables among the 4 ethnic cohorts were compared using χ^2 tests or Fisher's exact test for categorical variables and ANOVA tests for continuous variables. Fisher's exact test was used to compare the ApoE genotype and ApoE allele frequencies across groups. The age at onset of AD associated with each Apo ϵ allele was determined using either a 2-way ANOVA or a 1-way ANOVA, depending on whether ethnicity was adjusted. Student's *t* test was used to compare the effect of Apo ϵ 4 homozygosity and heterozygosity on age at onset. All statistical analyses were performed using SAS® statistical software, V9.2 for Windows.

Results

The clinical and demographic variables, age at onset and distribution of genotypes of ApoE are shown in table 1. Education of the 4 ethnic cohorts differed ($p < 0.001$), but was typical in terms of mean years for the populations in their geographical regions (table 1).

Distribution of the 6 ApoE genotypes differed among the 4 ethnic cohorts ($p < 0.001$, Fisher's exact test; table 1). Importantly, the mean ages at onset of the 4 ethnic cohorts did not differ ($p = 0.63$; table 1). Allelic frequency for ϵ 4 among the 4 ethnic cohorts differed ($p < 0.001$). It was highest in African Americans (50.0%) and lowest in Koreans (21.3%). The frequency of ϵ 3 ($p < 0.001$) was highest in Koreans (71.3%) and lowest in African Americans (45.8%). The ϵ 2 allelic frequency was low in all groups (but highest in Koreans), and the variation across ethnic groups did not quite reach statistical significance in this small study ($p = 0.06$; table 2).

In a 2-way ANOVA with age at onset as the outcome, and ethnicity and the ϵ 4 allele as factors, the ϵ 4 allele was significantly associated with younger age at onset ($p < 0.0001$), but ethnicity was not ($p = 0.66$). In a similar 2-way ANOVA using ethnicity and the ϵ 3 allele as factors, we found the ϵ 3 allele was significantly associated with older age at onset ($p < 0.0001$), but ethnicity was not significant. In a similar model, neither the ϵ 2 allele nor ethnicity was significantly associated with age at onset ($p = 0.20$, $p = 0.68$, respectively; table 3). For the within-group analysis, the effect of Apo ϵ 4 on age at onset was significant in Caucasians ($p < 0.0001$) and African Americans ($p < 0.05$), but not in Hispanics ($p = 0.07$) or Koreans ($p = 0.43$).

We performed three 2-way ANOVAs using age at onset as the outcome, and country and ApoE carrier status as factors in each model (table 4). The results showed that country was not significant in any of the 3 models. Having ϵ 4 and ϵ 3 were significant in the expected directions

Table 1. Clinical and demographic variables by ethnic group

	Hispanic	Caucasian	African American	Korean	p
Number of patients	55	1,083	84	87	
Gender, female, %	64	66	77	75	0.0694 ^a
Education, mean $\pm$ SD	12 $\pm$ 4.5	14 $\pm$ 5.5	13 $\pm$ 4.4	5 $\pm$ 4.6	<0.001 ^b
MMSE	16 $\pm$ 7.1	20 $\pm$ 6.5	15 $\pm$ 7.8	22 $\pm$ 4.8	<0.001 ^b
Age at onset	70 $\pm$ 9.1	71 $\pm$ 8.6	70 $\pm$ 10.0	71 $\pm$ 7.9	0.6314 ^b
ApoE genotypes, n (%)					<0.001 ^c
$\epsilon 4/\epsilon 4$	5 (9.1)	142 (13.1)	16 (19.0)	5 (5.7)	
$\epsilon 4/\epsilon 3$	27 (49.1)	500 (46.2)	48 (57.1)	23 (26.4)	
$\epsilon 4/\epsilon 2$	–	34 (3.1)	4 (4.8)	4 (4.6)	
$\epsilon 3/\epsilon 3$	23 (41.8)	362 (33.4)	13 (15.5)	47 (54.0)	
$\epsilon 3/\epsilon 2$	–	44 (4.1)	3 (3.6)	7 (8.0)	
$\epsilon 2/\epsilon 2$	–	1 (0.1)	–	1 (1.1)	

^a χ^2 test. ^b 1-way ANOVA test. ^c Fisher's exact test.

Table 2. The ApoE allelic frequency in different populations

	Hispanic	Caucasian	African American	Korean	p
Allele, n (%)					
$\epsilon 2$	–	80 (3.7)	7 (4.2)	13 (7.5)	0.0629 ^a
$\epsilon 3$	73 (66.4)	1,268 (58.5)	77 (45.8)	124 (71.3)	<0.001 ^a
$\epsilon 4$	37 (33.6)	818 (37.8)	84 (50.0)	37 (21.3)	<0.001 ^a

^a Fisher's exact test.

of association (both $p < 0.0001$), but having $\epsilon 2$ was not associated with age at onset. There was a significant difference in age at onset comparing $\epsilon 4$ homozygosity and $\epsilon 4$ heterozygosity for the combined cohorts ($p < 0.0001$ by Student's t test), again in the expected direction. When the ethnic groups were examined separately, this difference was significant in Caucasians ($p < 0.0001$) and Hispanics ($p < 0.01$), of borderline significance in African Americans ($p = 0.06$), and not significant among Koreans ($p = 0.36$).

Discussion

Our study confirmed the previously reported association between the presence of Apo $\epsilon 4$ and younger age at onset, as well as a dose effect of $\epsilon 4$ in the combined group. We also found that ethnicity was not an independent pre-

dictor of age of onset, which was consistently 70–71 years of age across the ethnic groups. However, the relationship between the Apo $\epsilon 4$ allele and age of onset was not separately apparent in Hispanics and Koreans in our study, possibly because of the low prevalence of the allele in these 2 groups, and/or the smaller group size for Hispanics, with a consequent loss of power to detect a significant association.

Since the reports that Apo $\epsilon 4$ was a risk factor for both late onset AD [1] and early onset AD [2], there have been many additional reports supporting ApoE $\epsilon 4$ as a genetic risk factor for late onset AD [3–5]. These reports are unequivocal in Caucasian populations, but this association with risk of AD is not as strong or consistent in African American and Hispanic populations [5, 7, 19]. Reports examining East Asians are relatively limited, involving small samples, and therefore inconclusive [3, 20–22].

Table 3. Age at onset of AD by ApoE allele status in 4 populations

	Hispanic (n = 55)		Caucasian (n = 1,083)		African American (n = 84)		Korean (n = 87)	
	AAO	n	AAO	n	AAO	n	AAO	n
$\epsilon 2$ Allele status ^a								
$\epsilon 2 = 2$			65.2 (0.0)	1			75	1
$\epsilon 2 = 1$ or 2	–		72.1 (8.8)	79	75.1 (7.6)	7	71.9 (5.9)	12
$\epsilon 2 = 1$			72.2 (8.8)	78	75.1 (7.6)	7	71.6 (6.1)	11
$\epsilon 2 = 0$	69.7 (9.1)	55	70.8 (8.6)	1,004	69.7 (10.1)	77	71.0 (8.2)	75
$\epsilon 3$ Allele status ^b								
$\epsilon 3 = 2$	68.5 (11.0)	23	71.6 (10.0)	362	73.1 (12.0)	13	71.5 (9.0)	47
$\epsilon 3 = 1$ or 2	70.4 (9.0)	50	71.4 (8.8)	906	71.3 (9.9)	64	71.1 (8.3)	77
$\epsilon 3 = 1$	72.0 (6.6)	27	71.3 (7.9)	544	70.8 (9.3)	51	70.5 (7.1)	30
$\epsilon 3 = 0$	62.6 (7.4)	5	68.5 (7.1)	177	66.5 (10.0)	20	71.2 (4.5)	10
$\epsilon 4$ Allele status ^b								
$\epsilon 4 = 2$	62.6 (7.4)	5	67.7 (6.5)	142	65.2 (10.4)	16	72.4 (4.6)	5
$\epsilon 4 = 1$ or 2	70.5 (7.4)	32	70.5 (7.7)	676	69.1 (9.5)	68	69.9 (6.5)	32
$\epsilon 4 = 1$	72.0 (6.6)	27	71.2 (7.9)	534	70.4 (9.0)	52	69.5 (6.7)	27
$\epsilon 4 = 0$	68.5 (11.0)	23	71.7 (9.9)	407	74.4 (11.3)	16	71.8 (8.6)	55

Values are means (SD). AAO = Age at onset.

^a Main effects of allele status and ethnicity nonsignificant by 2-way ANOVA.

^b Main effect of ethnicity nonsignificant, main effect of allele status significant ($p < 0.001$) by 2-way ANOVA.

Table 4. Age at onset of AD by ApoE allele status in the United States vs. Korean population

	United States (n = 1,222)		Korea (n = 87)	
	AAO	n	AAO	n
$\epsilon 2$ Carrier status ^a				
$\epsilon 2 = 2$	65.2	1	75	1
$\epsilon 2 = 1$ or 2	72.4 (8.7)	86	71.9 (5.9)	12
$\epsilon 2 = 1$	72.5 (8.7)	85	71.6 (6.1)	11
$\epsilon 2 = 0$	70.7 (8.7)	1,136	71.0 (8.2)	75
$\epsilon 3$ Carrier status ^b				
$\epsilon 3 = 2$	71.5 (10.1)	398	71.5 (9.0)	47
$\epsilon 3 = 1$ or 2	71.3 (8.8)	1,020	71.1 (8.3)	77
$\epsilon 3 = 1$	71.2 (8.0)	622	70.5 (7.1)	30
$\epsilon 3 = 0$	68.2 (7.5)	202	71.2 (4.5)	10
$\epsilon 4$ Carrier status ^b				
$\epsilon 4 = 2$	67.3 (7.0)	163	72.4 (4.6)	5
$\epsilon 4 = 1$ or 2	70.3 (7.9)	776	69.9 (6.5)	32
$\epsilon 4 = 1$	71.2 (7.9)	613	69.5 (6.7)	27
$\epsilon 4 = 0$	71.6 (10.0)	446	71.8 (8.6)	55

Values are means (SD). AAO = Age at onset.

^a Main effects of allele status and ethnicity nonsignificant by 2-way ANOVA.

^b Main effect of ethnicity nonsignificant, main effect of allele status significant ($p < 0.001$) by 2-way ANOVA.

When we compared the age at onset of $\epsilon 4$ homozygotes and $\epsilon 4$ heterozygotes, there was a significant difference, with younger onset in $\epsilon 4$ homozygotes in the combined population and in the 3 ethnic subgroups in the United States cohort. The effect of homozygosity was minimal in Koreans, who showed inverse age at onset for $\epsilon 4$ homozygotes and heterozygotes.

The failure to find a dose affect of $\epsilon 4$, as well as the lack of association of $\epsilon 4$ with age at onset overall in two of the ethnic groups, could have alternative explanations not related to sample size or allelic frequency variation. For example, lifestyle issues, such as dietary practices, with or without an interaction with Apo $\epsilon 4$ or cholesterol metabolism, could differ between the Eastern and Western regions or between Western subpopulations. Similarly, other genetic variations have recently been associated with age at onset, such as the copy number variation in the olfactory receptor gene, and these genetic variations may also differ by ethnicity [23]. The important hypothesis suggested by our study is that the genetic risk and age of onset modification conferred by an ApoE4 genotype may be modifiable by environmental factors or genetic background.

In comparison to the Apo $\epsilon 4$ allele, the Apo $\epsilon 3$ allele was significantly associated with later age at onset for the

combined group, while ethnicity was not significantly associated with age at onset for the combined group after adjusting for $\epsilon 3$ allele. Apo $\epsilon 3$ has been used as a base or neutral risk when it has been compared with $\epsilon 4$ for the calculation of odds ratios related to $\epsilon 4$ [3, 24]. It is often described as having a neutral effect or mildly beneficial effect in terms of risk for AD [10, 11], which is supported by the observation that the biological properties of $\epsilon 3$ fall between $\epsilon 2$ and $\epsilon 4$ [25]. We found that Apo $\epsilon 3$ is significantly associated with later age at onset of AD. As far as we know, this is the only clinical study which shows an association of Apo $\epsilon 3$ and later age at onset of AD.

This study has several limitations. First, the relatively small sample sizes for the 3 non-Caucasian ethnic groups reduced the power of the study to detect associations between ApoE genotype and age at onset. A larger study, with equal numbers from each ethnic group would be desirable for replication. In addition, the variable prevalence of some of the more rare alleles within the ethnic groups, such as the absence of the $\epsilon 2$ allele in Hispanics or the low prevalence of $\epsilon 4$ among Koreans, limits the conclusions that can be drawn from the study. Whether these differences reflect true ethnic variation or just random variation related to samples of convenience is not known. On the other hand, these findings are useful for sample size planning in future investigations on this topic. Second, this study uses a clinic-based population;

therefore, our findings may not translate to any given sample of population-based AD patients. However, the allelic frequencies of the Caucasian AD patients in our sample are consistent with previous large-scale meta-analyses, supporting the reliability of the sample. Third, the significance of AD symptoms as experienced by patients and inferred by caregivers may be different between ethnic groups, or could differ by genotype, which may bias estimation of age at onset. For all of these reasons, caution must be used in interpreting our age at onset results.

In summary, the ApoE polymorphism is significantly associated with age at onset of AD patients in a dose-related manner, with $\epsilon 4$ associated with earlier onset and $\epsilon 3$ associated with later onset. The background allelic frequencies, as well as the effects of these alleles on age of onset may differ for distinct ethnic groups or for different geographical regions. Other genetic or environmental factors may directly affect age at onset of AD or may modify the effect of ApoE genetic polymorphism in some or all populations.

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