The Apolipoprotein ϵ4 Allele in Patients with Alzheimer's Disease

Richard Mayeux, MD,*†‡§‖ Yaakov Stern, PhD,*‡§‖ Ruth Ottman, PhD,*†¶ Thomas K. Tatemichi, MD,*§ Ming-Xin Tang, PhD,*§ Gladys Maestre, MD,‡¶ Colleen Ngai, BS,** Benjamin Tycko, MD, PhD,‡¶ and Henry Ginsberg, MD††

Apolipoprotein E (APO-E) binds to the β-amyloid peptide and is present in senile neuritic plaques in Alzheimer's disease (AD). The ϵ4 isoform of APO-E has been associated with both sporadic and familial late-onset AD, implying a causal role. Among patients and control subjects similar in age, gender, and ethnic group from the New York City community of Washington Heights-Inwood, we found that the odds ratio (OR) for AD associated with homozygosity for APO-ϵ4 was 17.9 (95% confidence interval [CI], 4.6–69.8) and that associated with heterozygosity for APO-ϵ4 was 4.2 (95% CI, 1.8–9.5), compared with persons with other APO-E genotypes. The association was stronger among patients with sporadic disease (OR = 10.3; 95% CI, 3.4–31.1) than among those with a family history of dementia in a first-degree relative (OR = 0.9; 95% CI, 0.1–13.5). The association between APO-ϵ4 and AD did not differ according to age at onset (<65 vs ≥65), but appeared to vary across the 3 ethnic groups investigated (black, Hispanic, and white). Our data confirm the association between AD and APO-ϵ4 and support the hypothesis that the APO-ϵ4 allele either confers genetic susceptibility to AD or may be in linkage disequilibrium with another susceptibility locus. Ethnic variability in the allelic frequency of APO-ϵ4 in the elderly warrants further investigation.

Mayeux R, Stern Y, Ottman R, Tatemichi TK, Tang M-X, Maestre G, Ngai C, Tycko B, Ginsberg H. The apolipoprotein ϵ4 allele in patients with Alzheimer's disease. Ann Neurol 1993;34:752–754

The frequency of the ϵ4 allele of apolipoprotein E (APO-E) was found to be increased among patients with either sporadic or familial late-onset (onset of dementia after age 65) Alzheimer's disease (AD), compared with control subjects and patients with young-onset familial AD [1, 2]. Because the APO-E locus is on chromosome 19q13.2, near the site of linkage to late-onset familial AD [3], it may be a "susceptibility gene" for AD. We sought to replicate these observations in a community-based study of patients and control subjects from New York City.

Methods

Subjects and Setting

Blood for genomic DNA was obtained from 45 consecutively encountered patients meeting research criteria [4, 5] for probable AD who were participating in a community-based study of dementia during the period of March 1, 1993, to July 15, 1993. Age at onset and family history of dementia in first-degree relatives were obtained in structured interviews. Forty-three healthy elderly residents of the same community who participated in the study as control subjects were selected from a pool of 180 such individuals using frequency matching for age (within 5 years), gender, and ethnic group with the patients. Blood for genomic DNA was obtained from these individuals. All control subjects received the same interviews and clinical assessments as the patients and were found to be free of dementia and related disorders.

Genomic DNA Amplification and Restriction Isotyping of APO-E

APO-E genotypes were determined after isolating DNA from white blood cells and digesting with HhAI, using a method modified from that described by Hixson and Vernier [6]. All genotypes were determined without knowledge of patient or control subject status.

Data Analysis

Allele frequencies for patients with AD and control subjects were estimated by counting alleles and calculating sample proportions. Frequencies of APO-E genotypes in patients and control subjects were compared using the chi-square test. We estimated both simple and stratified odds ratios (OR) for AD associated with the presence of the APO-ϵ4 allele (homozygous and heterozygous) [7]. Odds ratios were also calculated using logistical regression to adjust for some covariates [8].

From the *Gertrude H. Sergievsky Center; the †Division of Epidemiology, Columbia University School of Public Health; the ‡Center for Alzheimer's Disease Research; the Departments of §Neurology, ‖Psychiatry, **Medicine, and ††Pathology, College of Physicians and Surgeons, Columbia University; and the ¶Epidemiology of Brain Disorders Research Department, New York State Psychiatric Institute, New York, NY.

Received Jun 10, 1993, and in revised form Aug 24. Accepted for publication Aug 24, 1993.

Address correspondence to Dr Mayeux, the G. H. Sergievsky Center, 630 West 168th St, Columbia University, New York, NY 10032.

Results

There were no differences between patients with AD and control subjects in age (AD, 67.2 ± 9.5; control subjects, 66.1 ± 8.9), gender (% women: AD, 64.4%; control subjects, 60.5%), and ethnicity (% white, black, and Hispanic, respectively: AD, 62, 16, and 22%; control subjects, 58, 14, and 28%).

APO-E 3/3 was the most frequent genotype among the control subjects (67.4%), whereas APO-E 4/3 was the most frequent genotype among patients (40%). The APO-ϵ4 allele frequency in patients was 0.34 and it was significantly higher than that in the control subjects (0.11; $\chi^2 = 14$; $p < 0.001$). Table 1 shows that the OR for AD associated with homozygosity for the APO-ϵ4 was 17.9 (95% CI, 4.6–69.8); the OR for heterozygosity was 4.2 (95% CI, 1.8–9.5). No control subject was homozygous for APO-ϵ4, whereas 5 patients with AD were homozygous (3 white, 1 black, and 1 Hispanic).

Among patients with AD, the APO-ϵ4 allele frequency was similar in whites, blacks, and Hispanics (0.34, 0.36, and 0.35, respectively; $\chi^2 = 2.6$; $p = 0.6$). Among control subjects, the allele frequency was significantly lower in whites (0.04) than in blacks (0.33) or Hispanics (0.13) ($\chi^2 = 11.1$; $p < 0.05$). This higher frequency of APO-ϵ4 among healthy black individuals has been previously described [9, 10]. Odds ratios for AD associated with APO-ϵ4 differed among the ethnic groups as noted in Table 2. The differences across the 3 ethnic groups in terms of the ORs for AD associated with APO-ϵ4 were of borderline statistical significance ($\chi^2 = 5.3$; $0.05 < p < 0.1$).

Twenty-one patients had onset of dementia before age 65, whereas the remaining 24 had onset at age 65 or older. The OR for AD associated with one or more APO-ϵ4 alleles was greater for patients with AD with onset before age 65 (OR = 7.4; 95% CI, 2.1–76.1) than for patients with onset at age 65 or older (OR = 3.4; 95% CI, 1.1–11.5) compared with control subjects, although these 2 ORs were not significantly different from each other.

The association between AD and APO-ϵ4 was stronger for patients with sporadic disease (OR = 10.3; 95% CI, 3.4–31.1) than for patients with a family history of dementia in a first-degree relative (OR = 0.9; 95% CI, 0.1–13.5), but the number of subjects with a family history of dementia was too small for definitive conclusions (AD = 9; control subjects = 4).

Table 1. Odds Ratio for Alzheimer's Disease Associated with Either Homozygosity or Heterozygosity for the APO-ϵ4 Isoform

Apolipoprotein E Genotype	Alzheimer's Disease	Control Subjects	Odds Ratio (95% CI)
(4,4)	5	0	17.9 (4.6–69.8)
(4,−)[a]	21	9	4.2 (1.8–9.5)
(−,−)	19	34	1.0 (reference)

[a]Under the genotype, (−) indicates any isoform other than ϵ4 (i.e., ϵ2 or ϵ3).

Table 2. Odds Ratios for Alzheimer's Disease Associated with One or More APO-ϵ4 Alleles by Ethnic Group

Ethnic Group	No. of Subjects: Alzheimer's Disease (n = 45)	No. of Subjects: Control Subjects (n = 43)	Odds Ratio (95% CI)
White			
≥1 ϵ4 allele[a]	16	2	15.3 (3.0–78.1)
0 ϵ4 allele	12	23	1.0 (reference)
Black			
≥1 ϵ4 allele	4	4	0.7 (0.1–6.4)
0 ϵ4 allele	3	2	1.0 (reference)
Hispanic			
≥1 ϵ4 allele	6	3	4.5 (0.7–27.7)
0 ϵ4 allele	4	9	1.0 (reference)
Mantel-Haenszel Estimate of Common Odds Ratio			5.2 (1.9–13.8)
Test for homogeneity (df = 2) $\chi^2 = 5.32$; $0.05 < p < 0.1$[b]			

[a]Number of subjects heterozygous or homozygous for APO-ϵ4.
[b]The test for homogeneity indicated that the difference in the odds ratios among the 3 ethnic groups was of borderline statistical significance.

Discussion

These results confirm earlier reports [1, 2] of an association between APO-ϵ4 and AD. However, our findings differ in that we found the association regardless of the age at onset of AD. Our results also raise the possibility that the association may differ across ethnic groups and may not be present in blacks.

APO-E is a lipoprotein involved in cholesterol transport in plasma; it is also produced and secreted in the brain by glial cells [11–14]. APO-E binds to the extracellular senile plaque, the neurofibrillary tangle, and at the site of amyloid angiopathy in patients with AD [2], as well as to amyloid plaques in patients with Creutzfeld-Jakob disease [15]. Because in vitro APO-E binds to the β-amyloid peptide, it may be involved in the intracellular and extracellular metabolism of amyloid [2]. Thus, finding an association between the APO-ϵ4 allele and AD implies that the ϵ4 isoform of apolipoprotein in particular could affect β-amyloid metabolism [2].

The previous association between APO-ϵ4 and AD was for both sporadic and familial late-onset forms. From an autopsy series and a clinic population, the APO-ϵ4 allele frequency for AD was 40%, compared with 15% in the control subjects, spouses, and a group of patients with early-onset AD [1]. The patients and

control subjects in these studies were of European ancestry; no other ethnic groups were investigated. Variability in the APO-E allele frequencies has been studied primarily in white populations, but available data indicate that the frequency of APO-ε4 allele for blacks from the United States and Nigeria [9, 10] can be as high as 30%. Data for Hispanic populations are limited; the frequency for Mexican-Americans was found to be similar to that in white populations [16]. The frequency of the APO-ε4 allele is also decreased among older individuals, possibly due to greater mortality from arteriosclerosis in ε4 carriers [12]. Thus, the low frequency of APO-ε4 among white control subjects in our study (0.04) might be explained, in part, by their advanced age, as well as by their selection to be free of AD. Cauley and associates [17] found the APO-ε4 allele frequency in healthy elderly populations to be approximately 9%, but AD was not investigated.

If the APO-ε4 allele has a direct role in increasing the risk of AD, then a large proportion of the population—between 4 and 30%, depending on the ethnic group—may be "at risk" by virtue of carrying at least one APO-ε4 allele. The 1% of the population homozygous for APO-ε4 [14, 17] may be at even greater risk, as shown by our data. Although our study was small, the lack of an association among blacks due to the higher frequency of the APO-ε4 allele among black control subjects may imply that APO-ε4 is in linkage disequilibrium with an AD susceptibility locus, rather than being a direct cause. Further investigation of the variability of the APO-ε4 allele in elderly populations and the mechanism by which the APO-ε4 allele augments susceptibility to AD seems warranted by these observations.

Support for this project came from the following federal grants: AG07232, AG10963, AG08702, HL36000, HL21007, and RR00645, and from the Charles S. Robertson Memorial Gift for Alzheimer's Disease Research from the Banbury Fund.

The authors would like to thank Drs A. M. Saunders, M. A. Pericak-Vance, and A. D. Roses of the Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Duke University Medical Center, Durham, NC, for sharing prepublication data with us so that this timely investigation could be done. We also appreciate the efforts of Arlene Lawton, RN, who obtained blood from the patients with AD.

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