Effect of Age, Ethnicity, and Head Injury on the Association between APOE Genotypes and Alzheimer’s Disease

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INTRODUCTION

The apolipoprotein E (APOE)-ε4 allele is neither necessary nor sufficient to cause Alzheimer’s disease (AD) because it develops in the absence of APOE ε4, 1, 2 and some persons escape the disease despite having an APOE ε4 allele. 3 Although the presence of the ε4 allele of the APOE gene has been consistently associated with an increased risk of AD, 4-20 it is apparent that the degree of risk may be modified by age, 10, 21 gender, 22 ethnic group, 2 certain risk factors, 23 and possibly other genes. 24

Roses et al. 11, 21 proposed that APOE genotypes have a direct influence on the age at onset of disease. In both familial and sporadic AD, an earlier age at onset among APOE ε4 homozygous and APOE ε4 heterozygous cases than among those cases with other APOE genotypes. Thus, it is possible that

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APOE genotypes strongly influence age at onset and that certain factors, both genetic and nongenetic, modify this effect by shifting the distribution curves.

In this review we will discuss demographic and putative risk factors that may modify (enhance or diminish) the association between APOE genotypes and AD.

METHODS

Subjects and Setting

All information was obtained from AD cases and controls residing in the same community and have been described in several earlier publications.\(^7,23,25\) Two different ascertainment methods were used to identify the study populations. Cases and controls were identified as part of a comprehensive community-based registry of all dementias in northern Manhattan and a random sample of Medicare recipients as previously described.\(^7\) The Columbia University institutional review board reviewed and approved this project. All individuals provided written informed consent. Both groups of cases and controls were followed over a four-year period.

Diagnosis of Alzheimer's Disease

The diagnosis of AD was based on standard research criteria.\(^26,27\) We previously reported the development of our diagnostic method and its relationship to the cultural and educational demographics of this community.\(^26,29\) The reliability and consistency of these assessments have also been described.\(^30\)

Age at Onset

Because we used a cross-sectional design with follow-up, a large number of cases identified with prevalent AD were included. We did not attempt to use an estimate of the age at onset because of the difficulty in substantiating this type of information. To improve validity, we used a more conservative method and censored the age at onset (left censored).\(^31-33\) We have recently described the method of estimating risks using this type of approach.\(^25\)

Ethnic Group

Ethnic group was classified by self-report using the format of the 1990 United States Census Bureau.\(^34\) This allows each person to identify him or herself as belonging to a particular racial or ethnic group. Each person is then asked whether or not he or she is of "Hispanic" origin.
APOE Genotyping

Genomic DNA was amplified by polymerase chain reaction (PCR) and subjected to CfoI restriction analysis using APOE primers and conditions modified from those as previously described by Hixson and Vernier. Genotypes were determined without knowledge of patient or control status.

Risk Factors and Family History

A structured interview was used to inquire about behaviors such as smoking, alcohol use, coffee consumption, dietary habits, antiperspirant use, insecticide exposure, exposure to household solvents, head injury, athletic activity now and in the past, previous hip or wrist fractures, and use of exogenous hormones. For several of the items, we attempted to determine the “dose” of the exposure with follow-up questions. A family history interview for AD and other neurological disorders in first-degree relatives (parents and full siblings) was obtained. The interviews were given in English or Spanish according to the preference of the patient’s informant or the control. The reliability of this interview has been reported.

Data Analysis

APOE allele frequencies were determined by counting alleles and calculating sample proportions. To compare across ethnic groups, APOE allele frequencies were calculated using all data from all controls and a 10% random sample of the total number of cases within each ethnic group to create a representative population. For comparison of cases and controls within and across ethnic groups, APOE allele frequencies were calculated for all subjects.

We used an iterative method to obtain the nonparametric maximum likelihood estimate of age at onset from the “self-consistent equation,” and then used a parametric approach to develop a proportional hazards regression model to estimate the relative risks of AD by APOE genotype.

Univariate and multivariate relative risks for AD associated with various APOE genotypes were derived from the proportional hazards model and both univariate, and multivariate odds ratios for AD associated with APOE ε4 were also calculated from logistic regression, adjusting for age, education, and ethnic group.

RESULTS

Demographics

Data were available for 276 cases and 405 healthy elderly unrelated controls. Cases and controls differed by gender (75% AD women vs. 66% control women, \( \chi^2 = 6.5, p < 0.01 \)). In all ethnic groups combined, cases
TABLE I. Demographics and APOE Allele Frequency among Patients with Alzheimer's Disease and Controls by Ethnic Groups

<table>
<thead>
<tr>
<th>Ethnic Groups</th>
<th>n</th>
<th>Age</th>
<th>Education</th>
<th>APOE-ε3</th>
<th>APOE-ε4</th>
<th>APOE-ε2</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>106</td>
<td>78 (7.6)</td>
<td>7.4 (4.0)</td>
<td>0.66</td>
<td>0.23</td>
<td>0.11</td>
</tr>
<tr>
<td>Controls</td>
<td>154</td>
<td>74 (5.8)*</td>
<td>9.4 (3.8)*</td>
<td>0.73</td>
<td>0.20</td>
<td>0.07</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>59</td>
<td>71 (9.1)</td>
<td>13.4 (4.2)</td>
<td>0.70</td>
<td>0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Controls</td>
<td>112</td>
<td>72 (8.5)</td>
<td>12.3 (4.0)*</td>
<td>0.84</td>
<td>0.09</td>
<td>0.07</td>
</tr>
<tr>
<td>Hispanic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD</td>
<td>140</td>
<td>77 (8.3)</td>
<td>4.8 (3.7)</td>
<td>0.74</td>
<td>0.19</td>
<td>0.07</td>
</tr>
<tr>
<td>Controls</td>
<td>219</td>
<td>73 (6.1)*</td>
<td>7.0 (4.3)*</td>
<td>0.83</td>
<td>0.10</td>
<td>0.07</td>
</tr>
</tbody>
</table>

NOTE: Allelic frequencies differed significantly between cases and controls within and across ethnic groups. See text for explanation.
* Denotes significant difference within each stratum (p < 0.05).

were older (AD 76.4 ± standard deviation [SD] 9.2 [range 46 to 96]) than controls (72.9 ± SD 6.7 [range 46 to 93], p < 0.05). African-Americans and Hispanics were older and less well educated than Caucasians (TABLE I). Cases also had less education (AD 7.4 ± 5.0 vs. controls 8.8 ± 4.5, p < 0.05) than controls.

**APOE Allele Frequencies**

The APOE allele frequencies also differed significantly in the three ethnic groups (χ² = 24.6, 4df, p < 0.0001). The frequency of APOE ε4 among African-Americans was higher with increasing age. Among African-Americans in the lowest quartile of age (below age 68) to the highest (age 77 and older) the APOE ε4 frequency was greater than 20%, whereas among Caucasians the frequency decreased from 16% to 7.5%. APOE allele frequencies did not differ by gender.

Among AD cases, the distribution of APOE alleles also differed across ethnic groups (χ² = 15.1, df4, p = 0.01), with higher frequencies of APOE ε4 in Caucasians (29%) than in African-Americans (24.7%) or Hispanics (18.6%) and with higher frequencies of APOE ε2 in African-Americans (12.1%) than in the other two ethnic groups (Caucasians 2.7%, Hispanics 6.6%; see TABLE 1).

**Relative Risk**

Specific APOE genotypes in cases and controls for each ethnic group are found in TABLE 2. The relative risk (RR) of AD among African-Americans homozygous for the APOE ε4 allele was significantly increased (RR = 3.3;
TABLE 2. Relative Risk of Alzheimer's Disease by APOE Genotypes in Cases and Controls by Ethnic Group

<table>
<thead>
<tr>
<th>Ethnic Groups</th>
<th>e3/e3</th>
<th>any ε2</th>
<th>any ε4</th>
<th>ε4/ε4</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>49 19</td>
<td>28</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>126 36</td>
<td>86</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.0 1.4; (0.8-2.6)*</td>
<td>0.6; (0.4-0.9)*</td>
<td>3.3; (1.6-6.8)*</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>30 1</td>
<td>24</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>108 15</td>
<td>44</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.0 0.3; (0.4-2.3)*</td>
<td>3.2; (1.8-5.8)*</td>
<td>5.3; (1.6-16.1)*</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>80 13</td>
<td>38</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>228 43</td>
<td>79</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.0 0.9; (0.5-1.8)*</td>
<td>1.5; (1.0-2.2)*</td>
<td>2.5; (1.1-5.8)*</td>
<td></td>
</tr>
</tbody>
</table>

Note: The number in each column represents the number of individuals with that specific genotype.

* 95% ci.

95% c.i. 1.6-6.8) compared with APOE ε3/ε3 genotypes. The risk associated with APOE ε4 heterozygosity was significantly decreased (RR = 0.6; 95% c.i. 0.4-0.9). For Caucasians, the relative risks associated with either APOE ε4 homozygosity (RR = 5.3; 95% c.i. 1.6-16.1) or heterozygosity (RR = 3.2; 95% c.i. 1.8-5.8) were significantly increased compared with APOE ε3/ε3 genotypes. Among Hispanics, the relative risks were increased for APOE ε4 homozygous individuals (RR = 2.5; 95% c.i. 1.1-5.8) and in APOE ε4 heterozygous individuals (RR = 1.5 95% c.i. 1.0-2.2) compared with APOE ε3/ε3 genotypes. Each analysis was repeated stratifying by gender and by including educational level as a covariate, but the results were identical.

We also examined the protective effect of APOE genotypes with one or more ε2 alleles using APOE ε3/ε3 genotypes again as the reference. The risk of disease associated with APOE ε2 was lower among Caucasians (RR = 0.3; 95% c.i. 0.4-2.3), but not African-Americans (RR = 1.4; 95% c.i. 0.8-2.6) or Hispanics (RR = 0.9; 95% c.i. 0.5-1.8).

**Head Injury**

In a subset of 113 patients with AD and 123 healthy elderly persons we used a case-control design to examine the joint effects of head injury and APOE genotypes. Thirteen patients (11.5%) with AD had a history of head trauma associated with loss of consciousness preceding onset of dementia, while 10 (8.1%) controls reported a similar injury. The odds ratio (OR) for AD associated with head injury was 1.5 (95% c.i. 0.5-3.5, p = 0.5). Using persons with neither a history of head injury nor an APOE ε4 allele as the
reference group, we estimated the OR for AD associated with both a history of traumatic head injury and the presence of at least one APOE ε4 allele (OR = 10.5; 95% c.i. 1.3-87.8), traumatic head injury alone (OR = 1.0; 95% c.i. 0.3-2.9), and the presence of APOE ε4 alone (OR = 2.0; 95% c.i. 1.1-3.5) adjusting for age by logistic regression. The OR for the joint effect of head injury and APOE ε4 exceeded that expected from the independent effects of both risk factors, suggesting a synergistic relationship.

**DISCUSSION**

We examined the effect of APOE genotypes on the relative risk of AD among three distinct ethnic groups residing in New York City. The APOE ε4/ε4 genotype was associated with a higher relative risk of AD in all ethnic groups. Among Caucasians and Hispanics, APOE ε4 heterozygosity was also associated with a significantly higher relative risk of AD compared with APOE genotypes without an ε4 allele. Among African-Americans, however, the increased risk was restricted to persons with the APOE ε4/ε4 genotype; APOE ε4 heterozygotes had a significantly lower relative risk of AD. Stratification by gender, family history of AD, or educational level did not change the association between APOE ε4 and AD in any ethnic group. There was only minimal evidence of a reduced risk for AD associated with APOE ε2 genotypes in Caucasians, and there was no decrease in risk for African-Americans or Hispanics.

A strong association between APOE ε4 and AD has been previously reported among African-Americans in Indiana. The APOE ε4 frequency among controls (0.12) was lower than that reported here. Differences in APOE allele frequencies by ethnic group are well established and there are regional differences among African populations.

The APOE ε4 allele frequency among the African-Americans in this study did not decline with age in contrast to the trend noted among Caucasians in the United States and Europe. The decline in the frequency of APOE ε4 with age in Caucasians has been attributed to mortality from heart disease because variations in the APOE locus affect cholesterol concentrations. APOE ε4 does not consistently predict cholesterol concentrations in persons of African origin. Thus it is possible that the APOE protein may be modified by other factors in African-Americans.

The data concerning head injury in this population suggest a synergistic relationship between an environmental risk factor and APOE ε4. In the absence of APOE ε4, the risk of AD associated with head injury was not elevated; thus, our results indicate that the effect of head injury on AD risk may be restricted to persons either homozygous or heterozygous for APOE ε4. The findings are most consistent with a model in which an environmental risk factor has no effect alone, yet it exacerbates the effect of genetic susceptibility. The findings further integrate the roles of β-amyloid and APOE ε4, suggesting that the APOE ε4 gene increases susceptibility to AD, which can be exacerbated by the effects of an environmental stimulus such as traumatic head injury.
Population-based prospective studies that address the demographic factors of age, ethnic group, gender, and socio-economic status should provide information concerning the risk of AD associated with APOE ε4 in these circumstances. Similarly, previous investigations of risk factors should be repeated to include APOE genotypes in order to appreciate the relationship between genetic and environmental factors in this disease.

SUMMARY

The association between apolipoprotein E (APOE)-ε4 and Alzheimer’s disease has been confirmed worldwide. We and others have observed a diminished association in the very old and among African-Americans compared to Caucasians and Hispanics in New York. In this review we describe a new method we developed to compare relative risks by APOE genotypes in an expanded cohort of cases and controls from three ethnic groups in a New York City community and discuss the association as between APOE ε4 and Alzheimer’s disease as modified by head injury.

Compared to persons with APOE ε3/ε3 genotypes, relative risk (RR) for Alzheimer’s disease associated with APOE ε4 homozygosity was similar across ethnic groups (African-American RR = 3.3; 95% c.i. 1.6-6.8; Caucasian RR = 5.3; 1.6-16.0; Hispanic RR = 2.5; 1.1-5.8). The risk was also increased for APOE ε4 heterozygous Caucasians (RR = 3.2; 1.8-5.8) and Hispanics (RR 1.5; 1.0-2.2) but not African-Americans (RR = 0.6; 0.4-0.9). Risk of AD was not significantly diminished for individuals in any group with APOE ε2/ε2 or -ε2/ε3 genotypes. A 10-fold increase in the risk of Alzheimer’s disease was associated with both APOE ε4 and a history of traumatic head injury, compared to a twofold increase in risk with APOE ε4 alone. Head injury in the absence of an APOE ε4 allele did not increase risk.

These results imply that in Alzheimer’s disease genotypic risk associated with APOE may be influenced by age, ethnicity, and certain enviromental factors.

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