Familial Alzheimer Disease Among Caribbean Hispanics

A Reexamination of Its Association With APOE

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Objectives: To reexamine the association between the apolipoprotein E ε4 allele (APOE ε4) and familial Alzheimer disease (AD), and to search for novel genes that may be associated with susceptibility in Caribbean Hispanic families with a history of AD.

Methods: Families were identified in Caribbean Hispanic communities in the greater New York City area, the Dominican Republic, and Puerto Rico. Each family in the study cohort included at least 2 living relatives with a history of dementia. All family members underwent neuropsychological testing and medical and neurological examinations to establish the presence or absence of dementia and to specify the type of dementia.

Results: Over a 2½-year period, 203 families were identified. Of these, 19 families had at least 1 family member with onset of dementia before age 55 years, with 8 of the 19 families showing an association with a previously unreported presenilin mutation. Multiple cases of AD were identified in 29 families. Overall, there were 236 affected sibling pairs with AD available for analysis. The average age at onset was 74 years. The presence of APOE ε4 was strongly associated with AD.

Conclusions: Both early-onset and late-onset familial AD occur in Caribbean Hispanics. In contrast to sporadic AD, late-onset familial AD among Caribbean Hispanics is strongly associated with APOE ε4. Future attempts to identify additional susceptibility genes should consider the effects of APOE ε4.
PARTICIPANTS AND METHODS

SOURCE POPULATION AND RECRUITMENT

Recruitment for the family study began in 1998. All patients in a population-based, community study of dementia in the Washington Heights–Inwood community, New York City, were eligible if they met inclusion criteria for our study. Patients were identified through registry information on 1330 individuals and a survey of 2250 Medicare recipients taken as a random sample from the community. Patients were also recruited from The Alzheimer Disease Research Center/Memory Disorders Center, from physicians’ private offices in the Department of Neurology, and from the General Medical Services, Columbia University, New York City. We used local newspapers, the local Hispanic radio station, and postings throughout Washington Heights–Inwood. Lectures were given at each of the 10 senior centers in the community. A system of recruitment was also set up in the Dominican Republic with the help of several local physicians, including the president of the Dominican Society of Geriatrics and Gerontology. Some investigators made annual visits to the Dominican Republic and Puerto Rico to fully assess all eligible families.

ASCERTAINMENT OF PROBANDS AND SIBLINGS

Once patients with AD were identified, their illnesses were documented with standardized neurological and neuropsychological evaluations. Then, structured family history interviews were conducted with available family members to determine whether patients had living siblings or relatives with the disease. We had previously established that reliability and validity of family history of AD among first-degree relatives was high.26 If the family history interview revealed a living sibling with suspected AD, that individual was also interviewed and examined. If a sibling of the proband had dementia, all other living siblings and available relatives were evaluated with the same examinations.

CLINICAL DIAGNOSIS

Medical and neurological examinations were completed for all family members, and patients were required to meet National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) research criteria for probable or possible AD.25 The Clinical Dementia Rating Scale (CDR)28 was used to rate the severity of dementia. A CDR score of 0 indicated no dementia; 0.5, questionable; 1, mild; 2, moderate; and 3, severe. Brain imaging and other laboratory studies were reviewed when available and offered when medically necessary to ensure full implementation of the NINCDS-ADRDA criteria. All patients in the Dominican Republic were offered the same diagnostic evaluations if deemed necessary.

The neuropsychological examinations used included a battery of tests modified for use with Spanish speakers.29,30 The tests included the Selective Reminding Test31; Benton Visual Retention Test, Matching and Recognition Memory32; the Orientation section of the Mini-Mental State Examination33; the Rosen Drawing Test34; the Boston Naming Test35; the Controlled Word Association Test36; the first 6 items of the Complex Ideational Material subtest of the Boston Diagnostic Aphasia Evaluation37; Wechsler Adult Intelligence Scale–Revised (Similarities section)38; and the Identities and Oddities subtest of the Mattis Dementia Rating Scale.39

Clinical data were reviewed at a consensus conference of neurologists and neuropsychologists. These methods and their development have been previously described.26,29,30 We included all those probands and siblings with probable AD, including those with CDR scores of 0.5. Our conservative definition of AD required a diagnosis of probable AD and a CDR score of 1 or more. Our liberal definition included participants with CDR scores of 0.5 or less who also met our neuropsychological criteria for probable AD but did not have functional impairment at the time of evaluation. Results from the 2 groups were compared for our study of APOE.

DNA COLLECTION AND APOE GENOTYPES

We collected blood from all patients with AD, their living siblings, and other family members. A modification22 of the methods described by Hixson and Vernier21 was used to determine APOE genotypes.

ASSOCIATION ANALYSIS

To examine whether the APOE e4 allele was transmitted more frequently in individuals with AD than by chance, we conducted a sibling transmission disequilibrium test (sib-TDT).41 Although the sib-TDT is conceptually comparable to the original transmission disequilibrium test (TDT),42 it allows testing of the transmission probability when parent genotype data are not available. As with the TDT, the sib-TDT determines association in the presence of linkage and avoids the problems of population stratification. To be used in the analysis, however, this method requires sibships to have at least 1 affected and 1 unaffected sibling; they should also have different genotypes.

RESULTS

During the first 2½ years of the project, 203 families were recruited. We divided extended families into multiple nuclear families. The majority of the families (81.3%) classified themselves as from the Dominican Republic, 24 (11.8%) were from Puerto Rico, and 14 (6.9%) came from elsewhere in the Caribbean. Overall, there were 728 individuals (241 men and 487 women) we examined and from whom we obtained DNA (Table 1). According to our conservative definition of AD, 306 participants (85 men and 221 women) had probable AD, and 218 were unaffected. Unaffected individuals were defined as those who were diagnosed without dementia at an age comparable to the probands. Of the remainder, 132 had CDR scores of 0.5, and 72 had other diagnoses of dementia. An ad-
ditional 63 individuals were coded as diagnosis unknown either because they had not been examined by us or because they were under age 40 years.

Nineteen families had at least 1 individual with onset of AD before age 55 years. We found a presenilin mutation in exon 7 in 8 (4%) of these families, and these 8 families were excluded from the sib-TDT analysis. In 29 families, multiple cases of AD were identified (Table 2). When we used a conservative definition of AD, there were 236 affected sibpairs. Eight families had 5 or more affected individuals, 4 families had more than 4 affected individuals, and 17 families had at least 3 affected family members. Sixty-three families had at least 2 affected individuals. The remaining 111 families had at least 1 affected individual who also had a mildly impaired relative. Finally, 81 families had at least 1 affected and 1 unaffected individual with APOE data, and 47 of these had at least 1 affected and 1 unaffected individual with different APOE genotypes.

Allele frequencies and APOE genotypes for both affected and unaffected individuals are provided in Table 3. The APOE ε4 allele was more likely to be transmitted among affected individuals than unaffected relatives (Table 4). The transmission probability of APOE ε2 was not significantly different from the null (Table 4). Because this study includes more than 200 families, the use of normal approximation is justified. This analysis is not a valid test of association, but it does represent a valid test of linkage.

We identified a large number of Caribbean Hispanic families with more than 1 individual with AD, including 8 with early-onset AD associated with a presenilin mutation. A much stronger association between AD and APOE ε4 was observed in these families than was previously found among elderly Caribbean Hispanics with late-onset sporadic AD. In a longitudinal cohort study using the APOE ε3/ε3 genotype as the reference, the relative risk of developing AD at age 90 years associated with 1 or more APOE ε4 alleles was 1.1, and 0.7 to 1.6 for Caribbean Hispanics with AD. However, the cumulative risk of AD at age 90 years among Caribbean Hispanics with an APOE ε4 allele was similar to that in whites, whereas in the absence of an APOE ε4 allele, Caribbean Hispanics were 2 times to 4 times more likely than whites to develop AD. The time-to-event variable was age at onset of AD, which required no further age adjustment.

The increase in risk was not related to differences in education or the presence of other risk factors such as a family history of AD-like dementia, suggesting that other genes or unknown factors may be involved.

There are at least 2 potential explanations for the differences in association between AD and APOE ε4 in these 2 studies. The community study included elderly individuals who were selected because they were residents of Washington Heights–Inwood and were registered Medicare recipients. The families in the current investigation were selected because they had at least 2 living family members with AD and were not from a single community. It is likely that the inclusion of individuals with familial AD enriched the association with APOE ε4, a point that has been noted previously. Compared with our previous study, the APOE ε4 allele frequency for controls in the current study was 40% higher (23.2% vs 14.1%); in cases the frequency increased by 54% (32.4% vs 14.8%). In addition, we previously found an increased risk for family members of patients with an APOE ε4 allele compared with other genotypes. Second, the average age at onset for AD in the current family study was 73.7 years, while the average age at onset was 81.4 years among Caribbean Hispanic patients residing in Washington Heights. Both results are consistent with what is already known about the effect of APOE ε4 on the age at onset of AD and its relationship to familial and sporadic forms of the disease.

Farrer et al. completed a worldwide meta-analysis of the relationship between APOE ε4 and AD described in numerous published and unpublished studies. They concluded that APOE ε4 was an important determinant of AD risk for men and women older than 60 years, but the association weakened after age 85 years. They also confirmed that APOE ε4 was strongly related to AD risk among whites and Asians. However, the relationship among African Americans and Hispanics remained inconsistent and weak in comparison, which supports our earlier findings. It is likely that the genetic influences for late-onset sporadic AD differ from those related to familial AD occurring earlier in life.

Studies examining the association between AD and APOE ε4 in Spain are consistent with results from

### Table 1. Demographics*

<table>
<thead>
<tr>
<th>No. of Subjects</th>
<th>Mean (SD)</th>
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<tbody>
<tr>
<td>Unaffected CDR 0.5 Other Dementia AD Total</td>
<td>Age at Onset of AD y</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Men 78</td>
<td>56</td>
</tr>
<tr>
<td>Women 140</td>
<td>74</td>
</tr>
<tr>
<td>Total 218</td>
<td>132</td>
</tr>
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</table>

*CDR 0.5 indicates a Clinical Dementia Rating Scale score of 0.5; AD, Alzheimer disease.

### Table 2. Pedigree Structures

<table>
<thead>
<tr>
<th>Pedigrees</th>
<th>No. of Families (n = 203)</th>
</tr>
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<tbody>
<tr>
<td>≥5 Affected siblings</td>
<td>8</td>
</tr>
<tr>
<td>4 Affected siblings</td>
<td>4</td>
</tr>
<tr>
<td>3 Affected siblings</td>
<td>17</td>
</tr>
<tr>
<td>2 Affected siblings</td>
<td>63</td>
</tr>
<tr>
<td>1 Affected mildly impaired</td>
<td>111</td>
</tr>
<tr>
<td>Families with ≥1 affected and ≥1 unaffected sibling and APOE data available*</td>
<td>81</td>
</tr>
<tr>
<td>Families with ≥3 affected and ≥1 unaffected with different genotypes†</td>
<td>47</td>
</tr>
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</table>

*APoE indicates apolipoprotein E.
†Some extended families had more than one sibship that met the conditions for the minimally required configuration.
other American and European studies. In contrast, Caribbean Hispanics from the Dominican Republic and Puerto Rico have a complex genetic heritage that differs from that of European Hispanics. First invaded by Spanish explorers, the Caribbean islands experienced political domination by Spanish, French, and British colonists for more than 500 years. Moreover, Africans captured as slaves were brought to the Caribbean during the same period. Our studies indicate that among Caribbean Hispanics from the Dominican Republic and Puerto Rico, sporadic AD is only weakly associated with APOE ε4, while familial AD is more strongly associated. Similar results have been found among Caribbean Hispanics from Cuba.24,25,49

Although it is possible that some as yet unidentified environmental factors have a causal role in the higher frequency of AD among Caribbean Hispanics, the weight of evidence suggests that AD is a predominantly genetic disorder. DNA from the families described here will be used in a genome-wide scan to search for the chromosomal location of other genes that may be associated with susceptibility to AD. The strong association between AD and APOE ε4 will require analyses to stratify for this allele.

Table 3. APOE Allele and Genotype Frequencies*

<table>
<thead>
<tr>
<th>APOE Allele</th>
<th>ε2</th>
<th>ε3</th>
<th>ε4</th>
<th>ε2/ε3</th>
<th>ε3/ε4</th>
<th>ε2/ε4</th>
<th>ε3/ε4</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>18 (3.0)†</td>
<td>382 (64.5)†</td>
<td>192 (32.4)†</td>
<td>12 (4.1)†</td>
<td>128 (43.2)†</td>
<td>6 (2.0)†</td>
<td>114 (38.5)†</td>
</tr>
<tr>
<td>Family controls</td>
<td>18 (3.0)</td>
<td>460 (74.0)</td>
<td>144 (23.2)</td>
<td>14 (4.5)</td>
<td>176 (57.9)</td>
<td>4 (1.3)</td>
<td>94 (30.2)</td>
</tr>
</tbody>
</table>

*Values given as No. (%). APOE indicates apolipoprotein E; AD, Alzheimer disease.

Table 4. APOE Transmission**

<table>
<thead>
<tr>
<th>APOE Allele</th>
<th>No. Alleles</th>
<th>Expected No. Alleles</th>
<th>Var (V)</th>
<th>Z*</th>
<th>P Value</th>
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<td><strong>Conservative Diagnosis of AD</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>3</td>
<td>79</td>
<td>93.39</td>
<td>12.92</td>
<td>3.87</td>
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<tr>
<td>4</td>
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<td>57.78</td>
<td>12.84</td>
<td>4.39</td>
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<td>2</td>
<td>5</td>
<td>6.82</td>
<td>1.86</td>
<td>0.97</td>
<td>.166</td>
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<td>97</td>
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<td>15.13</td>
<td>4.06</td>
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<tr>
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<td>87</td>
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<td>15.16</td>
<td>4.59</td>
<td>.0000023</td>
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<tr>
<td>2</td>
<td>6</td>
<td>8.96</td>
<td>1.87</td>
<td>1.14</td>
<td>.127</td>
</tr>
</tbody>
</table>

*A sibling transmission disequilibrium test 41 was used. APOE indicates apolipoprotein E; AD, Alzheimer disease.

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**Author contributions:** Study concept and design (Drs Romas and Mayeux and Mr Santana); acquisition of data (Drs Romas, Rondon, Estevez, Lantigua, Medrano, Torres, Tycko, and Mayeux, Mr Santana, and Mss Williamson and Ciappa); analysis and interpretation of data (Drs Romas, Lee, Stern, Tycko, and Mayeux and Ms Ciappa); drafting of the manuscript (Drs Romas, Lee, and Mayeux); critical revision of the manuscript for important intellectual content (Drs Romas, Rondon, Estevez, Lantigua, Medrano, Torres, Stern, Tycko, and Mayeux, Mr Santana, and Mss Williamson and Ciappa); statistical expertise (Drs Romas, Lee, Stern, and Mayeux); obtained funding (Drs Romas and Mayeux); administrative, technical, and material support (Drs Romas, Rondon, Estevez, Lantigua, Medrano, Torres, Tycko, and Mayeux, Mr Santana, and Mss Williamson and Ciappa); study supervision (Drs Romas, Lantigua, Tycko, and Mayeux); genotyping (Ms Ciappa).

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