The Absence of an Apolipoprotein ε4 Allele Is Associated with a More Aggressive Form of Alzheimer’s Disease

Yaakov Stern, PhD,*++ Jason Brandt, PhD,§ Marilyn Albert, PhD,|| Diane M. Jacobs, PhD,*+ Xinhua Liu, PhD,++ Karen Bell, MD,* Karen Marder, MD,*+ Mary Sano, PhD,*+ Steven Albert, PhD,*+ Caridad Del-Castillo Castenada, BA,* Fred Bylsma, PhD,|| Ben Tycko, MD,§ and Richard Mayeux, MD*++

We investigated the relationship between APOE genotype and rate of disease progression and survival in 99 patients with probable Alzheimer’s disease (AD) who were followed biannually for up to 6 years. Patients were stratified into two groups, those with and without at least one APOE ε4 allele. The rate of decline in modified Mini-Mental State Examination scores was slower, the presence of extrapyramidal signs was decreased, and the development of myoclonus occurred later among patients with APOE ε4 alleles compared with patients with other genotypes. Compared with patients without an APOE ε4 allele, the risk of mortality was also decreased in patients with at least one ε4 allele (RR = 0.38; CI = 0.17–0.84, p < 0.02). Because the decline in mental ability as well as the development of myoclonus and extrapyramidal signs are consistent manifestations of disease progression, our results imply that APOE ε4 is associated with a less aggressive form of AD.


Progression of Alzheimer’s disease (AD) in patients with one or two apolipoprotein E (APOE) ε4 alleles remains controversial. Survival with early-onset AD was better for patients with at least one ε4 allele than for patients with other alleles [1]. In late-onset AD, disease progression, as assessed by changes in global rating scales, was slower in APOE homozygotes than heterozygotes, and heterozygotes progressed more slowly than patients with other genotypes [2]. Corder and colleagues [3] found that the progression of AD, as measured by time from onset to death, was not strongly related to ε4 gene dose. The relationship between APOE genotype and rate of disease progression and survival in a cohort of prospectively followed patients with probable AD was investigated in 99 patients participating in a longitudinal study from three sites: New York, Boston, and Baltimore.

Subjects and Methods

Subjects
Subjects were all participants in the Predictors Study [4]. Two hundred thirty-six patients with probable AD (pAD) were recruited at Columbia University College of Physicians and Surgeons, Johns Hopkins University School of Medicine, and Massachusetts General Hospital. Details of inclusion and exclusion criteria, and recruitment methods have been previously described [4]. Each patient met National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria for pAD [5], with the exceptions described below. Severity of dementia was mild to moderate at study entry; all patients were required to have a modified Mini-Mental State Examination (mMMS) [6, 7] score of 30 or above (corresponding to approximately 16 on the standard Mini-Mental State Examination (MMSE) [8]).

Exclusion criteria included history or current clinical evidence of substance abuse, schizophrenia, schizoaffective disorder, or major affective disorder prior to the onset of intellectual decline; any electroconvulsive therapy (ECT) treatment within 2 years of recruitment, or 10 or more ECT treatments at any time; history or clinical signs of stroke or a Hachinski Ischemic Score [9] of 5 or more. The presence of central nervous system infection or other potential causes of dementia was also exclusionary. Patients with small subcortical lesions on computed tomographic or magnetic resonance imaging scan that were clinically and historically silent

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Address correspondence to Dr Stern, Sergievsky Center, 630 West 168th Street, New York, NY 10032.

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and were judged to be less than 2 cm in diameter were included. Diffuse symmetric periventricular lesions, such as those consistent with small-vessel ischemic change or infarcts, or indicative of cerebrospinal fluid absorption abnormalities, were not grounds for exclusion. Patients with cortical lesions of any size or location, or with focal cortical atrophy in a specific vascular distribution, were excluded.

Beginning in the sixth year of the study, available subjects contributed blood samples for APOE genotyping. In addition, analyses were conducted on frozen brain tissue from 12 participants. APOE genotypes were obtained for 99 of a pool of 150 subjects who were potentially available.

Procedures
All patients were followed at 6-month intervals. At each visit, the following data were obtained.

EXTRAPYRAMIDAL SIGNS AND MYOCLONUS. Selected items from the Unified Parkinson's Disease Rating Scale [10] were used to rate extrapyramidal signs (EPS); the reliability of the scale for use in pAD has been established [11]. Patients who had at least one sign rated as mild-moderate (2) were considered to have EPS. If a patient's signs were possibly or probably drug induced, then he or she was not included in the statistical analyses. The presence of myoclonus was also assessed. Because only 1 patient had myoclonus at the initial evaluation, the onset of myoclonus was treated as a study endpoint.

COGNITION. Cognitive function was examined using the mMMS [6, 7]. This instrument includes all items from the standard MMSE [8] and also includes the Wechsler Adult Intelligence Scale Digit Span subtest [12], and additional attention/calculation, general knowledge, language, and construction items. The maximum score on this test is 57.

Death
Family members usually informed us of patients' deaths. For patients who could not be contacted for follow-up or were otherwise lost to follow-up, death information was obtained as available through the National Death Index. Autopsies were performed whenever possible. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria [13] were used for the neuropathological diagnosis of AD.

APOE
APOE genotype was determined after isolating DNA from white blood cells and digesting the DNA with HhaI. The method for APOE genotyping by Hixson and Vernier [14] was modified slightly [15].

Data Analysis
For most analyses, patients were assigned to one of two groups, those with and without an APOE e4 allele. We considered subdividing patients with an e4 allele into homozygotes and heterozygotes but did not do so for most analyses for two reasons; ie, there were few deaths among the homozygous group (4 of 15), and exploratory analyses suggested that progression was similar for homozygotes and heterozygotes. Simple comparisons were done with t tests and one-way analyses of variance (ANOVAs) or \( \chi^2 \) tests as appropriate. A Kruskal–Wallis one-way ANOVA followed by a Kolmogorov–Smirnov (K-S) two-sample test was used to evaluate age at onset as a function of APOE status. These analyses were also calculated separately for patients with age at onset of more than 65 years. This cutoff point was selected because (1) it traditionally separates young and old age-at-onset patients, (2) several descriptions of the effect of APOE on onset have focused on late- as opposed to early-onset AD (for example, see Reference 16), and (3) there is a greater likelihood of other genetic contributions to AD with earlier onset.

To evaluate the decline in mMMS scores, we used a growth-curve modeling method that extends nonlinear growth-curve models [17] to characterize changes in prospectively collected data [18]. The goal of this procedure is to characterize the conditional average change in a score over the next 6 months based on the current score. The conditional average change is modeled with a function in a form similar to Von Bertalanffy's growth-curve model [17], which unifies monomolecular, logistic, Gompertz, and other models. It differs from standard growth-curve models in that it requires regular follow-up intervals and analyzes changes in scores as opposed to the scores themselves. The basic growth model is extended to include additional predictive variables, so that their effect on the conditional rate of change can be assessed. In this case, the effect of the presence or absence of an e4 allele was evaluated.

To evaluate the influence of APOE status on the relative risk (RR) of death or developing myoclonus, we used Cox analysis to evaluate relative survival from the patients' initial visit, correcting for estimated duration of illness. For mortality, gender and age and mMMS score at the initial visit were subsequently included as covariates.

Results
Demographics
Patient demographics are summarized in the Table. The mean age at the subjects' initial visit was 71.3 (±7.7) years and mean estimated duration of illness was 4 (±2.2) years. The mean mMMS score was 38.2 (±5.4).

The distribution of APOE genotypes was as follows: 15 of the patients were homozygous for the e4 allele, 38 were e4/e3, 3 were e4/e2, 4 were e3/e2, and 39 were homozygous for the e3 allele. APOE e4 allele frequency was 0.34, which is similar to that reported in other studies of AD populations.

Patients who were e4 homozygous or heterozygous, or who had other genotypes did not differ significantly in mean age, education, duration of illness, or mMMS score, or in the distribution of gender (see Table). Ethnic distribution differed significantly among the three groups (\( p < 0.01 \)); ie, all African-Americans and Hispanics had at least one e4 allele, while 43 of 90 whites did not have an e4 allele. Note, however, that only 9 of the subjects were not white.

At the time of these analyses, 2 patients were fol-
Table. Patient Demographics and Outcomes

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>4/-</th>
<th>4/4</th>
<th>No e4 Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>99</td>
<td>41</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>71.3 (7.7)</td>
<td>71.8 (7.3)</td>
<td>69.7 (4.4)</td>
<td>71.3 (9.0)</td>
</tr>
<tr>
<td>Education</td>
<td>13.6 (3.6)</td>
<td>13.8 (2.9)</td>
<td>13.9 (4.9)</td>
<td>13.3 (3.7)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>47.5</td>
<td>43.9</td>
<td>66.7</td>
<td>44.2</td>
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<tr>
<td>Ethnicity</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>White (%)</td>
<td>90</td>
<td>82.9</td>
<td>80.0</td>
<td>100</td>
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<tr>
<td>Black (%)</td>
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<td>4.9</td>
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</tr>
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<td>Hispanic (%)</td>
<td>5</td>
<td>12.2</td>
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<td>0</td>
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<td>Duration of illness</td>
<td>4.0 (2.24)</td>
<td>4.2 (2.6)</td>
<td>3.6 (1.8)</td>
<td>3.9 (2.0)</td>
</tr>
<tr>
<td>Age at onset (yr)</td>
<td>67.3 (7.8)</td>
<td>67.6 (7.7)</td>
<td>66.1 (4.0)</td>
<td>67.4 (8.9)</td>
</tr>
<tr>
<td>Young onset (%)</td>
<td>36</td>
<td>31.7</td>
<td>33.3</td>
<td>41.9</td>
</tr>
<tr>
<td>mMMS</td>
<td>38.2 (5.41)</td>
<td>37.3 (5.1)</td>
<td>40.7 (4.6)</td>
<td>38.5 (5.8)</td>
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<tr>
<td>EPS (%)</td>
<td>10.1</td>
<td>5.3</td>
<td>0</td>
<td>21.6</td>
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<tr>
<td>Myoclonus (%)</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td>Deaths</td>
<td>27 (27.2%)</td>
<td>6 (14.6%)</td>
<td>4 (26.6%)</td>
<td>17 (39.5%)</td>
</tr>
</tbody>
</table>

mMMS = modified Mini-Mental State Examination; EPS = extrapyramidal signs.

The patients included in this study were compared with those for whom APOE genotypes were not available. Those for whom genotypes were not available had a higher mean age at the initial visit (74.4 ± 9.5 years), a later age at onset (70.5 ± 9.8 years), and were more likely to be female (67.9%; all comparisons, p < 0.05). The two groups did not differ significantly in mean years of education, duration of illness, and mMMS scores at the initial visit, or in the distribution of ethnicity, young onset, EPS, or myoclonus.

Age at Onset
Mean estimated age at onset did not differ as a function of e4 status (see Table). To further evaluate this issue, we used a Kruskal-Wallis analysis to evaluate age at onset in patients with no, one, and two e4 alleles. The groups did not differ significantly, but the cumulative frequency of age-at-onset curves suggested a separation between the three groups for late-onset AD (Fig 1). The analysis was calculated separately for patients with onset above and below age 65. For patients with disease onset before age 65, there was no difference in the distribution of age at disease onset across the three groups. However, for patients with onset at 65 years or later, there was a significant difference across the three groups (χ² = 10.6, p < 0.01). In comparison with the no-e4 group, the homozygous e4 patients had an earlier age at onset (K-S: z = 1.4, p < 0.05), but heterozygotes did not differ significantly (K-S: z = 0.9, NS).

Rate of Progression
Mean decline in mMMS scores per 6-month interval was -2.49 (±1.57) in the e4 group and -2.90 (±2.12) in the no-e4 group (not significant). Results of the growth-curve modeling analysis are summarized in Figure 2. The model indicates that for each given score, the average conditional change of mMMS scores over a 6-month interval in the no-e4 allele group is larger than that group with at least one e4 allele. This coincides with empirically observed changes in that, despite some variability, the 6-month change from any particular mMMS score was generally larger in the no-e4 allele group than in the group with at least one e4 allele. Overall, the model indicates that the rate of decline in the group with at least one e4 allele is 0.72 times that for the group with no e4 alleles (p < 0.05). In evaluating the calculated model, 95% of the residuals were within 2 SD of the residuals and there was no systematic pattern on a residual plot.
Survival
There were 27 deaths; 17 of the 43 patients without an ε4 allele (39.5%) and 10 of the 56 patients with an ε4 allele (17.9%) died.

By using Cox analysis time from the patients' first visit to death was investigated, controlling for estimated duration of illness. The mortality RR was reduced in patients with at least one ε4 allele (RR = 0.38; 0.17–0.84, p < 0.02). The survival curves from the Cox analysis are displayed in Figure 3. This remained significant after adjusting for gender, age, and mMMS score at the initial visit (RR = 0.32; 0.12–0.74, p < 0.01). Results were unchanged after further adjustment for the site at which the patient was followed.

Extrapyramidal Signs and Myoclonus
Patients without an ε4 allele were significantly more likely to have EPS (OR = 7.03; 1.40–35.37, p < 0.01).

Eleven patients developed myoclonus during the follow-up period, 2 (3.3%) of the patients with at least one and 9 (20.9%) of the patients without an ε4 allele. A Cox analysis investigated time from the patients' first visit to onset of myoclonus (Fig 4) controlling for duration of illness at the initial visit. The RR was reduced in patients with at least one ε4 allele (RR = 0.11; 0.02–0.55). Results were unchanged after further adjustment for the site at which the patient was followed.
APOE ε4 allele was higher than that in patients with APOE ε4. While the presence of ε4 alleles was associated with an earlier age of onset of AD, adjusting for this variable indicated that risk of mortality was reduced for the patients with at least one ε4 allele. In a similar manner, when the time between the initial patient visit and death was considered, a reduced mortality RR among patients with ε4 alleles was evident.

The presence of the APOE ε4 allele is associated with earlier onset of AD. Thus, we had hypothesized that disease progression would be more rapid in patients with at least one ε4 allele. This hypothesis was bolstered by an observation that cognitive decline in nondemented elderly men was associated with the presence of ε4 in a dose-related fashion [19]. However, two other studies suggested that this was not the case for AD. In a study of autopsy-confirmed AD cases and affected members of AD families, there was no association between ε4 gene dose and survival [3]. Another study [2] estimated yearly MMSE score change in 62 late-onset (after age 70) patients by subtracting the current score from a norms-based estimated score and dividing by years of disease duration. They found rates of 4.7, 3.8, and 2.2 points per year in the −/−, ε4/−, and ε4/ε4 groups, respectively. They found similar results in 28 patients who were prospectively followed, but only 14 patients were followed for at least 12 months. Finally, a study of early-onset patients found that survival was best for patients with at least one ε4 allele, and worse for patients with an ε2 allele [1]. The current study differs from those reviewed in several ways: we did not focus specifically on either early- or late-onset cases, all of our patients were followed prospectively, and we considered both survival and rate of progression.

Growth-curve analysis indicated that the rate of decline of mMMS scores was more rapid in patients without an ε4 allele. In addition, patients without an ε4 allele were more likely to develop myoclonus and EPS. We used these clinical signs as endpoints in the current analyses, since we consider their presence or onset to be indicators of a transition to more advanced disease [20]. Several studies have demonstrated the prognostic value of myoclonus and EPS for mortality [21, 22] and support the association of myoclonus and EPS to more rapid disease course [23–27]. Nondemented elders with EPS are at increased risk for incident dementia [28], and EPS is associated with a particular neuropsychological profile in AD [29]. Thus, the association of EPS and myoclonus to APOE warrants further investigation.

There are several possible interpretations to the current findings. Frisoni and colleagues [2] suggested that patients without an ε4 allele are at greater risk for mortality and that this could underlie the greater increased frequency of the ε4 allele in patient prevalence studies. However, this explanation would not explain the observed earlier onset of AD in elders as a function of the number of ε4 alleles. Another explanation was raised by Corder and associates [3]; ie, the processes leading to the onset of AD differ from those that determine its clinical course. Thus, the presence of one or more ε4 alleles may confer greater risk for onset of AD, but have a different effect on the processes that determine rate of progression.

Patients included in this study were participants in the Predictors study [4], and were recruited from three medical research centers. A recent report demonstrated that patients seen at secondary and tertiary referral centers may differ demographically from a population sample in substantial ways [30]. The present sample may best be considered representative only of patients with pAD who are typically followed at research centers.

We allowed patients with clinically silent, small subcortical lesions into our cohort in recognition of the fact that such lesions are often observed in patients who otherwise meet rigorous criteria for pAD. We could not differentiate patients with and without these lesions on any clinical measures or with regard to rate of progression [31, 32].

Our data indicate that both cognitive and noncognitive manifestations of AD may be influenced by the presence of an APOE ε4 allele. Differences in the progression of AD as indicated by both slower decline in mental status scores and the later development of EPS and myoclonus, as well as reduced risk of death, indicate that the APOE ε4 allele may be associated with a less aggressive form of AD.

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References