Predictive Utility of Apolipoprotein E Genotype for Alzheimer Disease in Outpatients With Mild Cognitive Impairment

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Background: In cognitively impaired patients without dementia, the utility of apolipoprotein E (APOE) genotyping is unclear.

Objective: To evaluate the predictive utility of the APOE ε4 genotype for conversion to probable Alzheimer disease (AD).

Design: Naturalistic, longitudinal study.

Setting: Memory disorders outpatient clinic.

Patients: A total of 136 patients with memory complaints were determined to have mild cognitive impairment and were evaluated every 6 months. Fifty-seven age- and sex-matched healthy controls were evaluated annually.

Main Outcome Measures: Primary outcome measures included conversion to AD. Secondary outcome measures included change over time in Mini-Mental State Examination (MMSE) score and Selective Reminding Test (SRT) delayed recall score.

Results: The APOE ε4 allele was present in 25% of patients and 21% of healthy controls. During a mean±SD follow-up of 35.2±24.3 months, 35 of 136 patients converted to AD. APOE ε4 carrier status did not differ between converters (31%) and nonconverters to AD (23%, P = .3) and did not affect the time trend in MMSE or SRT scores in the entire sample. Four of 5 APOE ε4 homozygotes converted to AD compared with 7 of 29 heterozygotes (P = .02). In a Cox proportional hazards model stratified by age quartiles, after controlling for sex, education, MMSE score, and SRT delayed recall score, APOE ε4 increased the risk of AD in patients 70 to 85 years old (n = 57; risk ratio, 2.77; 95% confidence interval, 1.1-7.3; P = .03) but not in patients 55 to 69 years old (n = 79; P = .7).

Conclusions: APOE ε4 carrier status was associated with conversion to AD in older outpatients after controlling for known demographic and clinical risk factors, and APOE ε4 homozygosity was associated with increased risk of conversion to AD. However, APOE ε4 carrier status by itself did not predict cognitive decline or conversion to AD, indicating that APOE genotyping in patients with mild cognitive impairment may have limited clinical applicability for prediction of outcome.

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genotyping does not provide sufficient sensitivity and specificity to be a diagnostic test for AD, but when combined with clinical criteria it may improve specificity.22

The few longitudinal studies1,23 conducted in cognitively impaired outpatients without dementia have reported conflicting results regarding APOE genotyping in the prediction of AD, and physicians remain unclear about its utility in these patients. In a naturalistic, longitudinal study of cognitively impaired outpatients who did not meet clinical diagnostic criteria for dementia, the associations of the APOE ε4 genotype with baseline cognitive and functional measures and the predictive utility of the APOE ε4 genotype for cognitive decline and for the follow-up diagnosis of AD were examined.

SUBJECTS

Patients who presented with memory complaints to a memory disorders center, which included a research clinic and affiliated neurologists’ private offices, participated in a longitudinal study of putative early diagnostic markers of AD. Most (52%) were physician referred, 29% were self-referred, and 23% were referred by family, friends, or other sources. The research protocol was approved by the New York State Psychiatric Institute and Columbia Presbyterian Medical Center institutional review boards, and written informed consent was obtained from the patient and an informant (when available).

For patients, inclusion criteria were age of 40 years or older, intellectual impairment for 6 months or more but 10 years or less, and the diagnosis of “not demented” (Clinical Dementia Rating [CDR]=0) or “questionably demented” (CDR=0.5). Patients had a minimum modified Mini-Mental State Examination (MMSE) score of 40 or higher of 57 (Folstein MMSE score ≥22), but Spanish-speaking patients with 5 years or less of education and an MMSE score of 35 or higher (Folstein MMSE score ≥18) were included if they met all other inclusion and exclusion criteria. Neuropsychological testing screening guidelines were recall of 2 or fewer of 3 objects at 5 minutes on the MMSE, a delayed recall score more than 1 SD below norms on the Selective Reminding Test (SRT), or a Wechsler Adult Intelligence Scale–Revised (WAIS-R) performance IQ score of 10 points or more below the WAIS-R verbal IQ score. Patients without any of these deficits were eligible if they met all of the following criteria: subjective complaint of memory decline, informant’s confirmation of decline, and functional impairment (positive score on at least 1 of the first 8 items of the modified Blessed Functional Activity Scale).24

Exclusion criteria were a diagnosis of dementia, schizophrenia, schizoaffective disorder, or primary major affective disorder; electroconvulsive therapy within the past 6 months; current or recent (past 6 months) history of alcohol or other substance dependence (DSM-IV criteria); clinical, laboratory, and magnetic resonance imaging information to make a consensus research diagnosis. A similar approach was used for follow-up evaluations, as previously described.28,29 The diagnosis of dementia was based on DSM-IV criteria, and the diagnosis of possible or probable AD was based on criteria of the National Institute of Neurological and Cognitive Disorders and Stroke—Alzheimer’s Disease and Related Disorders Association.30 The consensus diagnosis was the primary outcome variable. The raters were blind to APOE results at all time points.

Subjective Memory Complaints

The self-report Memory Functioning Questionnaire31 was administered at baseline and annually to all subjects. The overall subjective rating (single item) and 4 subscales were analyzed: general frequency of forgetting, seriousness of forgetting, retrospective functioning (functioning compared with prior times), and mnemonics use.32

Functional Assessment Scale

The Pfeffer Functional Activities Questionnaire (FAQ)33 was given at baseline to all patients and controls and separately to informants of patients. On the basis of our prior work showing that informant report of function and not patient report predicts AD, only the informant report was used in the analyses.29 Each item was scored dichotomously (no difficulty or any difficulty), and the sum of items rated as “any difficulty” (range, 0-10) was analyzed. If a subject had never performed the task, the item was excluded from the total score.

Onset of Illness

In patients, the Onset of Illness interview was administered at baseline to an informant to determine the time of onset of memory decline and other deficits.34

APOE Genotyping

APOE genotyping was conducted using standard methods by which DNA was amplified by the polymerase chain reaction.35
Within 6 months of presentation, 2 patients with MCI were clinically diagnosed as having other neurologic disorders (corticobasal degeneration and amyotrophic lateral sclerosis presenting with frontal lobe deficits), and they were excluded. Patients scored lower than controls on the MMSE and SRT delayed recall, as expected, but were similar in other features (Table 1). In the 136 patients with MCI, 57% had a CDR of 0.5 (questionable dementia) and 43% had a CDR of 0 (no dementia).

### APOE GENOTYPE

The APOE genotype distribution in patients was as follows: 3,3, 67%; 2,3, 8%; 2,4, 0.7%; 3,4, 21%; and 4,4, 4%. The APOE genotype distribution in controls was as follows: 3,3, 60%; 2,3, 16%; 2,4, 2%; 2,2, 4%; 3,4, 18%; and 4,4, 2%. APOE ε4 carrier status did not differ between patients (25%) and controls (21%; \( P = .6 \)) or between patients with a CDR of 0 (28%) and a CDR of 0.5 (23%; \( P = .5 \)). In patients (and controls), the presence of APOE ε4 was not associated with age, sex, education, ethnicity, Pfeffer FAQ score, or baseline MMSE or SRT delayed recall score (Table 2). In patients, the global rating of subjective memory and the subscale scores for general frequency of forgetting, mnemonics use, and retrospective functioning were not related to APOE ε4 status. For the subscale of seriousness of forgetting, APOE ε4 noncarriers rated their memory as worse compared with APOE ε4 carriers (\( t_{124} = 2.3; P = .02 \)).

### FOLLOW-UP

Of the 136 patients who were followed up, 16 had dropped out by the 3-year follow-up time point: 11 patients had died and 5 patients had refused follow-up. There were 22 dropouts by the 5-year follow-up time point: 13 patients had died, 2 patients had lost contact, and 7 patients had refused further follow-up. In the 3 brain autopsy specimens obtained, neuropathologic analysis confirmed the clinical diagnosis. Four of the 35 patients with incident AD had other neurologic conditions (1 had Parkinsonism, 1 had Lewy body disease, and 2 had cerebrovascular disease).
brovascular disease) that were deemed contributing factors. All 35 incident AD cases were retained for statistical analyses. Of the 57 controls, 2 progressed to a CDR of 0.5 but none converted to dementia during follow-up.

Thirty-four of 78 patients with a baseline CDR of 0.5 compared with 1 of 58 patients with a baseline CDR of 0 converted to AD during follow-up (Fisher exact test; \( P < .001 \)). Converters were older and had lower baseline MMSE scores than nonconverters (Table 3). Mean±SD duration of follow-up was 35.2 ± 24.3 months; converters \(( n = 35)\) were diagnosed as having AD a mean±SD of 21.6 ± 15.6 months after initial evaluation, and nonconverters \(( n = 101)\) were followed up a mean±SD of 46.9 ± 25.1 months. Controls were followed up a mean±SD of 52.9 ± 24.9 months.

In patients, APOE \( \varepsilon 4 \) carrier status did not differ between converters (31%) and nonconverters (23%; \( \chi^2 = 1.0; \ P = .3 \)). Four of 5 APOE \( \varepsilon 4 \) homozygotes converted to AD compared with 7 of 29 heterozygotes who converted to AD during follow-up (Fisher exact test; \( P = .03 \)).

In the entire sample of 193 subjects (patients plus controls), APOE \( \varepsilon 4 \) carrier status did not predict conversion to AD in survival analyses (log-rank test; \( P = .2 \)). In a Cox proportional hazards model that controlled for sex \(( P = .2)\), age \(( P = .04)\), baseline MMSE score \(( P < .001)\), and SRT delayed recall score \(( P < .001)\), APOE \( \varepsilon 4 \) status tended to predict conversion to AD \(( \text{risk ratio}, 2.0; 95\% \text{CI}, 0.9-4.4; \ P = .08 \)). Since no control converted to AD and the primary aim was to evaluate the predictive utility of APOE \( \varepsilon 4 \) in patients with MCI, further analyses were restricted to patients.

In survival analyses conducted in the 136 patients, APOE \( \varepsilon 4 \) status did not predict conversion to AD \(( \text{log-rank test}, \ P = .2)\). In a Cox proportional hazards model that controlled for sex \(( P = .4)\), age \(( P = .02)\), education \(( P = .5)\), baseline MMSE score \(( P = .004)\), and SRT delayed recall score \(( P < .001)\), APOE \( \varepsilon 4 \) status tended to predict conversion to AD \(( \text{risk ratio}, 2.0; 95\% \text{CI}, 0.9-4.4; \ P = .08)\). In a Cox model that included age, APOE \( \varepsilon 4 \), and the age-by-APOE \( \varepsilon 4 \) interaction, after controlling for sex, education, and baseline MMSE and SRT delayed recall scores, the age-by-APOE \( \varepsilon 4 \) interaction tended to predict AD \(( P = .06)\), with the predictive effect of APOE increasing with baseline age.

To further assess the baseline age-dependent effect of APOE, patients younger than 55 years \(( n = 17)\) were excluded because none converted to AD. The remainder of the patient sample was dichotomized around the median age of 70 years. Without controlling for other variables, APOE \( \varepsilon 4 \) status did not predict AD in patients 55 to 69 years old \(( \text{log-rank test}, \ P = .35)\) but tended to predict AD in patients 70 to 85 years old \(( \text{log-rank test}, \ P = .08)\) (Figure). In a Cox proportional hazards model stratified by age quartiles, after controlling for sex, education, and MMSE and SRT delayed recall scores, the presence of APOE \( \varepsilon 4 \) significantly increased the risk of AD in patients 70 years or older \(( \text{risk ratio}, 2.77; 95\% \text{CI}, 1.1-7.3; \ P = .03)\) but not in patients 55 to 69 years old \(( P = .8)\). When age at onset replaced age in these analyses, the results were essentially unchanged.

In another set of Cox models in the patient sample, APOE \( \varepsilon 4 \) status was not a significant predictor of AD after including the covariates of baseline subjective memory complaints global score, Pfeffer FAQ score, or baseline SRT delayed recall score in each model. Restricting the sample to white patients \(( n = 109)\) and controlling for age, sex, education, and MMSE and SRT delayed recall scores, the presence of APOE \( \varepsilon 4 \) tended to predict conversion to AD \(( \text{risk ratio}, 2.5; 95\% \text{CI}, 1.0-6.3; \ P = .06)\) in another Cox model. In similar Cox analyses with the same covariates in older \(( \geq 70 \text{ years})\) white patients, APOE \( \varepsilon 4 \) significantly increased the risk of AD \(( \text{risk ratio}, 3.5; 95\% \text{CI}, 1.1-11.2; \ P = .04)\). Restricting the patient sample to a baseline CDR of 0.5 \(( n = 78)\) and controlling for age, sex, education, MMSE score, and SRT delayed recall score in another Cox model, APOE \( \varepsilon 4 \) status was not a significant predictor of AD \(( \text{risk ratio}, 1.8; 95\% \text{CI}, 0.8-4.0; \ P = .15)\). In the subsample with a CDR of 0.5, APOE \( \varepsilon 4 \) homozygosity \(( 3 \text{ homozygotes}; \text{thus, power was limited})\) was not a significant predictor \(( P = .4)\) in Cox analyses that controlled for the same covariates.

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**Table 3. Characteristics of Converters and Nonconverters to Alzheimer Disease**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Converters (n = 35)</th>
<th>Nonconverters (n = 101)</th>
<th>( P ) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female, No. (%)</td>
<td>21 (60)</td>
<td>55 (54)</td>
<td>.5</td>
</tr>
<tr>
<td>Age, mean ± SD, y</td>
<td>73.0 ± 7.2</td>
<td>65.0 ± 10.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Education, mean ± SD, y</td>
<td>14.0 ± 4.7</td>
<td>15.4 ± 4.1</td>
<td>.08</td>
</tr>
<tr>
<td>Ethnicity, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>74</td>
<td>78</td>
<td>.7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>17</td>
<td>13</td>
<td>.6</td>
</tr>
<tr>
<td>Black</td>
<td>3</td>
<td>6</td>
<td>.4</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>3</td>
<td>.8</td>
</tr>
<tr>
<td>MMSE score, mean ± SD</td>
<td>26.4 ± 1.9</td>
<td>28.1 ± 1.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>SRT delayed recall score, mean ± SD</td>
<td>2.9 ± 2.0</td>
<td>6.2 ± 2.8</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Apolipoprotein E ( \varepsilon 4 ) carrier, %</td>
<td>31</td>
<td>23</td>
<td>.3</td>
</tr>
</tbody>
</table>

Abbreviations: MMSE, Mini-Mental State Examination; SRT, Selective Reminding Test.

*Determined using \( \chi^2 \) test (categorical variables) or 2-tailed \( t \) test (continuous variables).

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**Figure.** Effect of apolipoprotein E \( (\text{APOE} \varepsilon 4) \) carrier status on survival time (Kaplan-Meier curve) to Alzheimer disease (AD) in 136 outpatients with mild cognitive impairment. For patients with a baseline age of 55 to 69 years, the presence of APOE \( \varepsilon 4 \) tended to reduce the time to AD \(( \text{log-rank test}, \chi^2 = 2.98; \ P = .08)\).
The main analyses were performed again by classifying the sample into APOE ε2 carriers and noncarriers instead of APOE ε4 carriers and noncarriers. APOE ε2 was not significant in any analysis for the prediction of or protection against AD.

In GEE analyses with repeated measures in the entire patient sample, there was no time trend in MMSE or SRT delayed recall scores, with and without controlling for age, sex, and education. In the subsample of 35 converters to AD, with the analyses restricted to the 3 years of follow-up before diagnostic conversion to AD, APOE ε4 status did not affect the decreasing linear time trend in MMSE scores, with and without controlling for age, sex, and education. In these 35 converters, patients with APOE ε4 showed a greater decline (P = .03) in the decreasing linear time trend for SRT delayed recall scores (P < .001) after controlling for age, sex, and education.

**COMMENT**

The prevalence of APOE ε4 carrier status did not differ between patients and controls. In patients, APOE ε4 carrier status was not associated with measures of subjective memory, in contrast to some reports. APOE ε4 carrier status by itself predicted neither conversion to AD nor decline in memory or global cognition in the patient sample. These results are consistent with some but not all studies of this type. Although APOE ε4 carrier status did not predict cognitive decline in the patient sample, it was associated with the declining linear trend in SRT delayed recall score in the subsample that converted to AD. However, APOE ε4 status did not add significantly to the prediction obtained from the SRT delayed recall score, in contrast to another report.

The APOE ε4 effect on conversion to AD appeared to increase with age (Table 4), and the analyses indicated that this effect remained after controlling for relevant covariates in the entire patient sample and in white patients only. The literature on APOE ε4 and age is complex, and recent epidemiologic data suggest that APOE ε4 is associated with an earlier age at onset but that this association is absent after the ninth decade of life. In our sample, the oldest patient was aged 85 years at baseline, thus limiting the evaluation of APOE ε4 effects in the very old.

Study limitations included the moderate sample size and the paucity of minorities (n = 27) to evaluate this subgroup separately or to assess ethnic differences. Autopsy was performed on only 3 of 13 patients who died; APOE ε4 appears to provide moderate to strong sensitivity and specificity for the autopsy diagnosis of definite AD. Therefore, the primary outcome of AD may have contained classification error, and some current nonconverters may convert to AD with longer follow-up. This limitation was tempered by using expert raters who used strict diagnostic methods and by using repeated-measures analyses (GEEs) on cognitive scores that were statistically powerful and eliminated the subjective element in diagnosis. The setting of the outpatient academic center limits generalizability to other types of settings, but to improve clinical relevance, the inclusion and exclusion criteria identified a broad group of patients with MCI. However, these differ from the narrower criteria for MCI used in other longitudinal clinical studies. Nonetheless, the number of converters to AD (n = 35) was large enough to assess predictors of conversion to AD.

Four of 5 patients with APOE ε4 homozygosity converted to AD, consistent with the literature on AD and in samples without dementia. The APOE ε4 homozygotes were important determinants of the overall effects in survival analyses, since the APOE ε4 carrier effect became less significant after homozygotes were excluded. There was no APOE ε2 effect, and data about the possible protective role of APOE ε2 against AD are conflicting.

In summary, APOE ε4 carrier status by itself did not predict cognitive decline or conversion to AD, indicating that APOE genotyping in patients with MCI may have limited clinical applicability. However, APOE ε4 carrier status was associated with conversion to AD in older outpatients after controlling for known demographic and clinical risk factors, and APOE ε4 homozygosity was associated with increased risk of conversion to AD. Overall, the results indicate that assessment of the APOE genotype should remain an important research tool in the investigation of patients with MCI and AD.

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